CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-266

21-267

MICROBIOLOGY REVIEW

REVIEW FOR HFD-590
OFFICE OF NEW DRUG CHEMISTRY
MICROBIOLOGY STAFF
MICROBIOLOGIST'S REVIEW #1 OF NDA

June 27, 2001

A. 1. NDA **21-267**

SPONSOR Pfizer Global Research and Development
Eastern Point Road
Groton, Connecticut 06340

2. PRODUCT NAMES: VFEND™ I.V. (Voriconazole for injection)

3. DOSAGE FORM AND ROUTE OF ADMINISTRATION: Lyophilized powder in a 20 mL vial for reconstitution in sterile Water for Injection and further diluted (to 2 – 5 mL) before intravenous infusion with a parenteral solution. Each vial contains 200 mg of voriconazole.

4. METHOD(S) OF STERILIZATION: [redacted]

5. PHARMACOLOGICAL CATEGORY: Antifungal

6. DRUG PRIORITY CLASSIFICATION: 1S

B. 1. DATE OF INITIAL SUBMISSION: November 17, 2000

2. DATE OF AMENDMENT: n/a

3. RELATED DOCUMENTS: DMF [redacted]

4. ASSIGNED FOR REVIEW: December 21, 2000

C. REMARKS: This electronic NDA was forwarded for consultation to the Microbiology Staff along with paper volumes 1.1 and 1.2. DMF [redacted] was provided for the information relating to the manufacture of the intravenous presentation of the product. A letter of authorization from [redacted] (dated August 29, 2000) was provided in the NDA. An oral tablet presentation is also part of the NDA but is not part of this review. The primary goal date for this NDA is September 17, 2001.

D.. CONCLUSIONS: The application is recommended for APPROVAL.

David Hussong, Ph.D.

cc:

HFD 160/Consult File
HFD 590/Division File
HFD 590/CSO/Saliba
HFD 590/Chemist/Holbert
HFD 805/D. Hussong

Drafted by: D. Hussong, 06/27/2001
R/D initialed by: P. Cooney

Filename, d:\nda\21-267rv1.DOC

Microbiology Review

Division of Special Pathogen and Immunologic Drug Products

(HFD-590)

NDA# 21-266 and 21-267

Reviewer : Linda Gosey
Correspondence Date : 11-17-00
CDER Receipt Date : 11-21-00
Review Assigned Date : 11-22-00
Review Complete Date: 11-02-01

Sponsor: Pfizer Pharmaceuticals
50 Pequot Avenue
New London, CT 06320

Submission Reviewed: Original

Drug Category: Antifungal

Indication: Treatment of invasive Aspergillosis infections, esophageal Candidiasis, fungal infections in febrile neutropenic patients and the treatment of rare pathogens and subjects who are refractory to or intolerant to other therapies.

Dosage Form: Oral Tablets and Intravenous

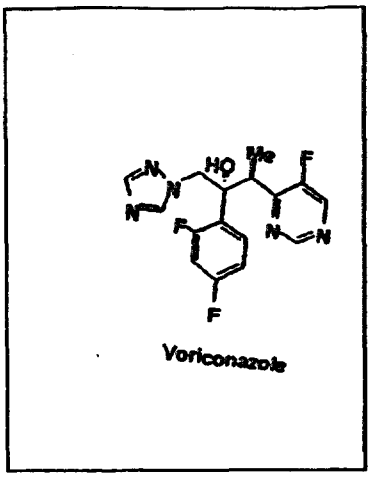
Product Names:

a. Proprietary: Vfend

b. Nonproprietary: Voriconazole; UK-109,496

c. Chemical: (2R,3S)-2-(2,4-difluorophenyl)-3-(5-fluoro-4-pyrimidinyl)-1-(1H-1,2,4-triazol-1-yl)-2-butanol

Structural Formula:



Supporting Documents: IND [redacted]

Executive Summary:

Pfizer is seeking approval of voriconazole for the treatment of esophageal candidiasis, presumptive fungal infections in febrile neutropenic patients, invasive Aspergillus infections and the treatment of rare pathogens in subjects who are refractory to or intolerant to other antifungal therapies. This review will focus on the pre-clinical data describing the microbiologic activity of voriconazole against *Candida, Aspergillus, Fusarium* and *Scedosporium* species as well as the clinical microbiology data from the esophageal candidiasis and invasive aspergillosis clinical trials.

A number of pre-clinical in vitro studies were reviewed and the data from those studies demonstrated that voriconazole has varying activity against *Candida* species. Preclinical in vitro susceptibility testing, employing the National Committee for Clinical Laboratory Standards (NCCLS) M27-A method, demonstrated that in general voriconazole minimum inhibitory concentration (MICs) were comparable to itraconazole MICs when tested against the same *Candida* isolates. In vitro time kill studies demonstrated that neither voriconazole nor itraconazole were cidal against the various *Candida* species evaluated. No pre-clinical in vitro studies were conducted to determine the frequency at which drug resistance development occurred in *Candida, Aspergillus, Fusarium* or *Scedosporium* species.

Preclinical in vitro susceptibility results showed good correlation between fluconazole susceptible (MIC $\leq$ 12.5 µg/ml) and resistant (MIC $\geq$100 µg/ml) isolates of *C. albicans* and voriconazole MIC values of $\leq$ 0.09 µg/ml and $\geq$ 0.39 µg/ml, respectively. Itraconazole and voriconazole MICs were higher for non-albicans species including *C. glabrata, C. parapsilosis, C. keyfr, C. lusitaniae, C. guilliermondii* and *C. tropicalis.*

Several investigators assessed the pre-clinical in vitro activity of voriconazole. While individual MIC values may have varied from one study to another the MIC results demonstrated that for *Aspergillus* species voriconazole MIC values were similar to those of itraconazole, indicating comparable activity against these moulds. However, it should be noted that the majority of the preclinical studies evaluated voriconazole against *A. fumigatus* and *A. flavus* only.

Employing the NCCLS M38-P method voriconazole MICs ranged from
for the *Aspergillus fumigatus* and *A. flavus* isolates tested. Growth inhibition time kill studies demonstrated that the cidal activity of voriconazole was not as great as that seen with amphotericin B but was greater than that seen with equal concentrations of itraconazole when tested against the conidial form of *A. fumigatus*. However, cidal activity defined as 99.9% killing, was not obtained by voriconazole in these studies. When the cidal activity of voriconazole and itraconazole were tested against the filamentous stage of growth of various *Aspergillus* species, 8-16 fold higher concentrations of both drugs were required to kill the hyphal phase of growth of the *Aspergillus* isolates. From the limited pre-clinical microbiology data regarding *Aspergillus* species, it is unclear if drug resistance development occurs or if cross-resistance is an issue.

The sponsor conducted a limited number of experiments assessing the antifungal activity of voriconazole when used in combination with other antifungal agents. From a single experiment it appears that voriconazole does not interact with either amphotericin B or 5-fluoro-cytosine (5FC) when tested against *C. albicans*. However, when tested against a single isolate of *A. fumigatus* it appeared that voriconazole activity was reduced when used in combination with 5FC and possibly with amphotericin B. These results should be viewed as preliminary as additional studies should be performed to accurately assess the activity of voriconazole when used in combination with other antifungal agents.

Voriconazole demonstrated in vivo activity against acute systemic *A. fumigatus* and *C. albicans* infections in immunocompetent and immunocompromised guinea pigs. In newly infected immunocompetent guinea pigs high doses of voriconazole were comparable to itraconazole in prolonging survival and reducing the fungal burden of *A. fumigatus* in kidney and lung tissue. Voriconazole also demonstrated activity in immunocompromised guinea pigs infected with an *A. fumigatus* strain demonstrating reduced activity to

itraconazole (itraconazole MIC of 3.1 µg/ml). Voriconazole was active against pulmonary infections in immunocompromised guinea pigs due to *Aspergillus fumigatus*. The in vivo activity of voriconazole was not studied against other *Aspergillus* species.

Voriconazole exhibited activity in newly infected normal and immunocompromised guinea pigs infected with fluconazole susceptible and resistant strains of *C. albicans*. Voriconazole also demonstrated varying effects with respect to the reduction of the mycological burden in the kidney of immunocompromised guinea pigs with acute systemic infections produced by *C. krusei* and *C. glabrata* isolates. The in vivo activity of voriconazole was not measured against other *Candida* species.

In the esophageal candidiasis clinical trial 305, the NCCLS M27-A susceptibility testing method was employed to determine fluconazole, itraconazole and voriconazole MICs. For the microbiologic assessment only per protocol subjects with an efficacy assessment at the end of treatment and who had microbiologic data at baseline were evaluated. There were 76/115 (66%) subjects in the voriconazole arm and arm 96/141 (68%) subjects in the fluconazole arm that met these criteria. A satisfactory response at the end of therapy was obtained in 60/76 (79%) voriconazole subjects and 67/96 (70%) fluconazole treated subjects. Greater than 94% of the subjects in both treatment arms with a satisfactory response were due to *C. albicans* with voriconazole MICs $\leq$0.19 µg/ml. All of these isolates were also susceptible to fluconazole and itraconazole.

At the end of therapy there were 7/76 (9%) and 14/96 (15%) per protocol subjects that had an unsatisfactory response in the voriconazole and fluconazole arms, respectively. The majority of these subjects continued to be infected with *C. albicans* where the isolates had no significant change in the fluconazole or voriconazole MIC values as compared to the pre treatment samples. Two subjects in the voriconazole arm and 4 subjects in the fluconazole arm developed a new fungal infection due to a different *Candida* species at the end of therapy. At the end of therapy drug resistance development occurred in one subject with *C. albicans* in the voriconazole arm and 2 subjects in the fluconazole arm (1 *C. albicans*, 1 *C. glabrata*). Drug resistance development was evident in these isolates, as there was a 16-64 fold increase in fluconazole, itraconazole and voriconazole MIC values between the pre treatment isolate and the end-of-therapy isolate.

Relapses, 30 days post therapy, occurred in 12% and 15% of the subjects in the voriconazole and fluconazole arms, respectively. All of the relapses in the voriconazole arm were due to *C. albicans* with voriconazole MICs $\leq$0.78 µg/ml. In the fluconazole arm 60% of the relapses were due to *C. albicans*, 13% due to *C. glabrata* and 27% due to mixed cultures containing *C. albicans* and other *Candida* species. Varying voriconazole, itraconazole and fluconazole MIC values were observed for the other yeast recovered during this study which included *C. glabrata*, *C. krusei*, *Saccharomyces* species, and *C. famata*.

There were insufficient numbers of infections due to *C. glabrata* or *C. krusei* isolates to draw decisive conclusions. The observed *in vitro* results suggest that voriconazole has reduced activity against *C. glabrata* isolates. It was also observed that *Candida albicans*

isolates that were resistant to both itraconazole and fluconazole also had significantly higher voriconazole MICs, suggesting cross-resistance.

In the invasive aspergillosis clinical trial 307/602, 50/77 (65%) and 41/68 (60%) MITT subjects in the voriconazole and amphotericin B arms, respectively, had a single species of *Aspergillus* recovered from a clinically relevant site. In the voriconazole arm 43/50 (86%) of these documented fungal infections were due to *A. fumigatus* of which 21/43 (49%) had a satisfactory response at week 12 of the study. Only 27/41 (66%) MITT subjects in the amphotericin B arm had documented fungal infections due to *A. fumigatus*. A total of 5/27 (19%) subjects had a satisfactory global response at week 12.

A significant number of subjects in the voriconazole arm 27/77 (35%) and amphotericin B arm 27/68 (40%) had multiple fungi (yeasts and moulds) recovered from clinically relevant sites. More subjects in the voriconazole arm had *A. fumigatus* and *C. albicans* or *A. fumigatus* and *C. glabrata* isolated from clinically relevant sites, of which, a satisfactory response of 50% and 0% was observed, respectively. Subjects in the voriconazole and amphotericin B arms with *A. fumigatus* and other moulds isolated from clinically relevant sites had satisfactory response rates of 0 and 43%, respectively.

The ability to accurately assess the efficacy of voriconazole in the MITT subjects with culture confirmed infections due to non-fumigatus species of *Aspergillus* was difficult due to the low incidence of infections due to each species. However, the overall trend with respect to a satisfactory global response suggested reduced voriconazole activity against infections caused by non-fumigatus *Aspergillus* species, including *A. flavus, A. terreus* and *A. nidulans*.

Voriconazole, itraconazole and amphotericin B susceptibility testing was performed on *Aspergillus* isolates sent to the reference laboratory. Greater than 90% of the *A. fumigatus* isolates had voriconazole MICs between 0.125-0.25 µg/ml employing the NCCLS M38-P method. Itraconazole MICs for these isolates ranged from The majority of the non-fumigatus species of *Aspergillus* isolated during these clinical trials had voriconazole and itraconazole MICs between
respectively, a 2-4-fold increase over those determined for the *A. fumigatus* isolates.

Cross-resistance between voriconazole and other oral antifungal azoles was not observed with the *Aspergillus* isolates recovered during this clinical trial. It was also observed that for the *Aspergillus* isolates studied a significant rise in voriconazole MICs did not occurred while subjects were on antifungal therapy.

Because there are no approved antifungal susceptibility testing methods for the filamentous fungi and voriconazole breakpoints have not been established for these fungi it is impossible to correlate voriconazole MIC results and clinical outcome.

In the historical controlled clinical trial 304, *A. fumigatus* was the most common *Aspergillus* species recovered from the MITT subjects with culture confirmed disease. Fifty three percent of the subjects with *A. fumigatus* as the sole pathogen isolated from a clinically relevant site had a satisfactory response. Satisfactory response rates were reduced to 29% in subjects who had *A. fumigatus* and another fungal organism isolated

from clinically relevant sites. Too few cases of confirmed invasive Aspergillosis due to non-fumigatus *Aspergillus* species were observed to adequately characterize the activity of voriconazole against these species. However, the general trend with respect to global response showed reduced activity against non-fumigatus species of *Aspergillus* to that observed against infections due to *A. fumigatus* alone.

There are sufficient preclinical and clinical data showing that voriconazole is active against azole susceptible strains of *C. albicans* and *A. fumigatus*. However, there was insufficient preclinical information and clinical microbiology results to adequately characterize the activity of voriconazole against non-albicans *Candida* and non-fumigatus *Aspergillus* species.

For *Scedosporium apiospermum, Fusarium solani* and *Fusarium oxysporum* isolates in vitro data demonstrated reduced voriconazole activity, suggesting that some isolates of each species may be less susceptible to voriconazole than *Aspergillus* or *Candida* isolates. In addition, consistently higher MICs were noted for *Scedosporium prolificans* isolates indicating that this pathogen may be even more difficult to treat with voriconazole. When cidal levels were determined it was found that significantly higher levels of voriconazole, $\geq$16 µg/ml, were required to kill *Scedosporium* and *Fusarium* species, further indicating that it may be more difficult to eliminate these organisms than *Aspergillus fumigatus*. Pfizer conducted one in vivo experiment where voriconazole at 5 and 10 mg/kg reduced the fungal burden in brain, lung, liver, kidney and spleen of immune competent guinea pigs infected with *Scedosporium apiospermum.* In vivo studies further characterizing the activity profile of voriconazole against the other rare moulds have not been performed to date. Due to the small numbers of these rare moulds studied both in vitro and in the clinical trials it is difficult to accurately correlate voriconazole MIC values and clinical outcome of subjects with infections due to these organisms. It is strongly recommended that the sponsor continue to collect clinical and microbiologic data on subjects with culture confirmed disease due to these rare pathogens and are treated with voriconazole.

In an effort to better understand the spectrum of activity of voriconazole the sponsor should collect clinical and microbiologic information on subjects infected with non-albicans *Candida,* non-fumigatus *Aspergillus* species, *Fusarium* species and *Scedosporium* species. The sponsor should also continue to characterize the cross-resistance and drug resistance patterns of voriconazole, as this information is critical in determining the appropriate treatment of patients with *Candida* and *Aspergillus* infections. As part of a phase IV commitment the sponsor should annually submit this information to the FDA.

Background:

Voriconazole, like fluconazole and itraconazole, is an antifungal azole. It inhibits the cytochrome P-450 dependent 14α-lanosterol demethylase enzyme that is responsible for the synthesis of ergosterol, a major component necessary for the integrity of the fungal cell wall. As such, the spectrum of activity of voriconazole is expected to be similar to the other antifungal azoles.

To fully characterize the microbiologic activity of voriconazole, three constituents were assessed in detail in the review of the pre-clinical and clinical microbiologic data. The first constituent was the activity profile of voriconazole against fungal species known to have reduced activity against either fluconazole or itraconazole, such as *C. glabrata, C. krusei* and *A. terreus*.

The second microbiologic constituent studied was cross-resistance between voriconazole, fluconazole or itraconazole. Cross-resistance between fluconazole and itraconazole has been reported for *Candida* species. However, the extent of the cross-resistance observed can either be partial or total, as various mechanisms of resistance have been observed with itraconazole and fluconazole. While voriconazole breakpoints have not been established significant changes in voriconazole minimum inhibitory concentrations (MICs) were closely studied and were used to indicate drug resistance development. When possible changes in MIC patterns for the three azoles were compared.

The third microbiologic constituent examined in this NDA review was drug resistance development. Drug resistance development has been well documented with subjects who have received long-term fluconazole therapy. It was critical to determine if this same phenomenon occurred with voriconazole. The majority of the data pertaining to cross resistance and drug resistance development will be discussed in the Clinical Review Section under the Medical Officer's Review of the NDA. Please refer to those sections for review of the clinical microbiology data.

Preclinical Microbiology Summary:

Mechanism of Action:

Voriconazole is a triazole antifungal agent. The mechanism of action is the same as for the other approved antifungal azoles, i.e. fluconazole and itraconazole. Voriconazole has been shown to inhibit the cytochrome P-450 dependent 14α-lanosterol demethylase enzyme that is responsible for the removal of the methyl group on the C14 site of lanosterol. Inhibition of this enzyme results in the depletion of ergosterol, a major component necessary for the integrity of the fungal cell wall, and the accumulation of the sterol precursor compounds. In vitro studies demonstrated that voriconazole is more selective for fungal cytochrome P-450 enzymes than for various mammalian cytochrome P-450 enzyme systems evaluated.

While the mechanism of action of voriconazole is the same as the other approved antifungal azoles, it is still necessary to characterize the activity of voriconazole against the different fungal pathogens. This is essential because the composition and content of the different sterols in the cell walls of fungal organisms can vary, thus potentially altering the activity profile and cross resistance pattern of voriconazole from genus to genus and even species to species.

Mechanisms of resistance:

There is no single mechanism of resistance for the antifungal azoles. The principal mechanisms, at the molecular level, contributing to antifungal azole resistance include modifications to the ergosterol biosynthesis pathway, molecular changes to the ERG gene and over expression of efflux pumps through either CDR genes of ABC transporters or the MDR1 gene.

In Vitro Activity:

Voriconazole has demonstrated in vitro activity against *Aspergillus* and *Candida* species. In 1998 Johnson et al. published data comparing the in vitro activity of voriconazole to that of itraconazole and amphotericin B against filamentous moulds (JAC, 1998, 42:741-745). *A. fumigatus, A. flavus, Fusarium solani* and *S. apiospermum* isolates were tested in addition to other moulds. MICs were determined employing a modified NCCLS M27-A microdilution method. After 48 hours of incubation, when the MICs were read, the microtiter wells demonstrating no growth were plated out and incubated for an additional 48 hours to determine the minimum lethal concentrations (MLC). The MLC was defined as the lowest drug concentration where 95% of the inoculum was killed. The *A. fumigatus, A. flavus* and *S. apiospermum* isolates tested had voriconazole MIC_{90}s of 0.5µg/ml. MLCs were 0.5, 1.0 and >16 µg/ml, respectively (See Table 1). The MIC_{90} and MLC for *Fusarium solani* were 4 and 16 µg/ml, respectively. These data show that significantly higher concentrations of voriconazole are required to kill *S. apiospermum* and *Fusarium solani* isolates as opposed to *Aspergillus flavus* or *A. fumigatus* isolates. The data also show that for the *Aspergillus* isolates tested, itraconazole and voriconazole MLCs were comparable to their MICs.

Table 1

In-vitro susceptibility of moulds

Table. In vitro susceptibilities of 110 isolates (ten of each species listed) to voriconazole, itraconazole and amphotericin B

		MIC (mg/L)			MLC (mg/L)		
Species	Antifungal agent	range	MIC_{50}	MIC_{90}	range	MLC_{50}	MLC_{90}
Absidia corymbifera	voriconazole		16	16		>16	>16
	itraconazole		0.25	0.5		2	>16
	amphotericin		0.25	0.25		0.25	1
Aspergillus flavus	voriconazole		0.5	0.5		0.5	1
	itraconazole		0.25	0.25		0.25	0.5
	amphotericin		1	1		1	1
Aspergillus fumigatus	voriconazole		0.25	0.5		0.25	0.5
	itraconazole		0.5	0.5		2	2
	amphotericin		0.5	0.5		1	4
Cladophialophora bantiana	voriconazole		≤0.03	0.12		0.5	1
	itraconazole		0.06	0.12		0.25	1
	amphotericin		0.25	0.5		1	1
Exophiala dermatitidis	voriconazole		0.12	0.25		0.25	0.5
	itraconazole		0.25	0.5		0.25	0.5
	amphotericin		0.5	1		0.5	1
Fonsecaea pedrosoi	voriconazole		0.06	0.06		0.06	0.06
	itraconazole		0.12	0.25		0.12	0.25
	amphotericin		1	1		1	2
Fusarium solani	voriconazole		2	4		8	16
	itraconazole		>16	>16		>16	>16
	amphotericin		1	2		1	2
Phialophora parasitica	voriconazole		0.25	0.25		1	2
	itraconazole		1	2		>16	>16
	amphotericin		1	2		1	2
Rhizopus arrhizus	voriconazole		8	16		16	>16
	itraconazole		1	2		1	>16
	amphotericin		0.25	0.25		0.25	1
Scedosporium apiospermum	voriconazole		0.25	0.5		>16	>16
	itraconazole		1	4		>16	>16
	amphotericin		2	8		>16	>16
Sporothrix schenckii	voriconazole		1	>16		16	>16
	itraconazole		2	4		16	>16
	amphotericin		2	4		2	4

The inhibitory and cidal activities of three antifungal agents were determined using NCCLS susceptibility testing methods and time kill curves, respectively (Manavathu et al., 1998, AAC 42:3018-21). Table 2 shows amphotericin B, itraconazole and voriconazole MICs for various *Aspergillus* and *Candida* isolates tested. For the *Aspergillus* isolates evaluated voriconazole and itraconazole MICs ranged from [redacted] respectively. The voriconazole MICs were comparable to the itraconazole MICs for the various *Aspergillus* isolates tested.

Voriconazole and itraconazole MICs against the *Candida* isolates ranged from [redacted] respectively. However, the MICs for both drugs were 8-32

fold higher for the non-albicans species versus *C. albicans*. The MIC data suggest that the inhibitory effect of voriconazole is comparable to that of itraconazole. In addition, the activity of both azoles is greater against *C. albicans* than non-albicans *Candida* and *Aspergillus* species.

TABLE 2. Susceptibilities of microorganisms tested[a]

Microorganism	Source[b]	MIC (μg/ml)[c]		
		AMB	ITZ	VCZ
A. fumigatus W73355	DMC	0.5	0.5	0.5
A. fumigatus F55064	DMC	0.5	0.25	1
A. fumigatus H52950	DMC	1	0.25	0.5
A. fumigatus T52654	DMC	0.5	0.25	0.5
A. fumigatus Z28996	DMC	1	0.5	1
Aspergillus niger S11338	DMC	4	4	1
A. niger F51729	DMC	1	4	1
A. niger I71775	DMC	1	4	1
A. niger W7884	DMC	2	0.25	0.25
A. niger T57275	DMC	2	0.5	2
Aspergillus flavus I65850	DMC	0.5	0.25	1
A. flavus I65680	DMC	8	0.25	0.5
A. flavus W69597	DMC	4	0.25	0.5
A. flavus S47511	DMC	4	0.5	0.25
A. flavus W72335	DMC	4	0.25	0.25
Aspergillus sp. M65388	DMC	0.5	0.5	0.5
Aspergillus sp. I35077	DMC	1	0.5	0.25
Aspergillus sp. R26451	DMC	2	4	0.5
C. albicans 90028	ATCC	0.02	0.031	0.015
Candida guilliermondii 9390	ATCC	0.25	0.5	0.25
Candida kefyr 40438	DMC	0.5	0.25	0.5
Candida parapsilosis CM205.95	DMC	0.5	0.125	0.125
Candida krusei LR061.90	DMC	0.5	0.125	0.125
Candida stellatoidea OW575.90	DMC	0.125	0.125	0.125
Candida tropicalis 44508	ATCC	0.5	0.25	0.25
Candida glabrata 33554	ATCC	0.5	0.5	0.5

[a] Results shown are from a typical experiment. Each value represents the mean of two independent determinations. MIC determinations were repeated at least once, and the results were within ±1 dilution.
[b] DMC, Detroit Medical Center; ATCC, American Type Culture Collection.
[c] AMB, amphotericin B; ITZ, itraconazole; VCZ, voriconazole.

Growth inhibition studies were conducted to determine the cidal activity of voriconazole. Figure 1 shows the time killing curves of amphotericin B, itraconazole and voriconazole against the conidial form of an *A. fumigatus* isolate. At 24 hours a 5μg/ml concentration of itraconazole and voriconazole killed 87-96% and 95-99% of the cells, respectively. Amphotericin B produced the greatest cidal activity. Neither azole obtained a killing activity of 99.9%; the quantity of killing traditionally designated to define cidal activity. It is also of interest to note that the killing activity was determined at 24 hours not 48 hours. The cidal response should have been determined at 48 hours, as some of the fungal elements may not have had adequate time to sporulate.

Figure 1

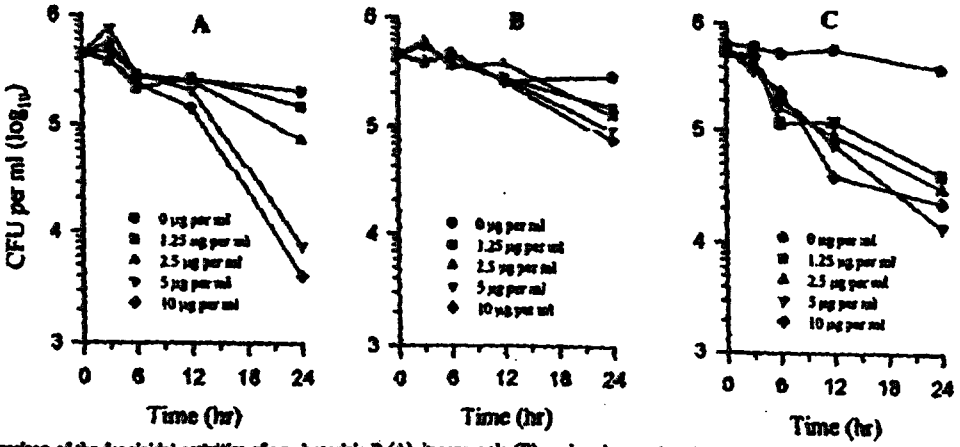


FIG. 1. Comparison of the fungicidal activities of amphotericin B (A), itraconazole (B), and voriconazole (C) against *A. fumigatus* WT5000. Each point represents the mean of two independent determinations. Experiments were repeated three times with similar results; the data shown here are from a typical experiment.

Cidal activity was also determined for various *Candida* species. Table 3 clearly demonstrates that neither voriconazole nor itraconazole are cidal against any of the *Candida* species tested.

TABLE 3. Fungicidal or fungistatic activities of amphotericin B, itraconazole, and voriconazole against *Aspergillus* and *Candida* species[a]

Microorganism	10^5 CFU/ml at T_0	10^5 CFU/ml at T_{24} (% change)[b]		
		AMB	ITZ	VCZ
A. fumigatus (n = 5)	8.52 ± 2.67	0.071 ± 0.091 (−99.2)	0.195 ± 0.297 (−97.7)	0.096 ± 0.055 (−98.9)
A. niger (n = 5)	4.19 ± 1.85	0.074 ± 0.097 (−98.2)	0.541 ± 0.349 (−87.1)	0.224 ± 0.140 (−94.7)
A. flavus (n = 5)	9.06 ± 4.78	0.173 ± 0.288 (−98.1)	0.317 ± 0.223 (−96.5)	0.376 ± 0.243 (−95.9)
Aspergillus sp. (n = 3)	5.57 ± 5.08	0.081 ± 0.123 (−98.5)	0.207 ± 0.284 (−96.3)	0.052 ± 0.023 (−99.1)
C. albicans	7.35 ± 0.68	0.00005 (−100)	65.3 ± 5.2 (+788.4)	50 ± 7.2 (+580.2)
C. guilliermondii	12.3 ± 4.2	0.00005 (−100)	16.3 ± 7.7 (+32.5)	19.2 ± 15.6 (+56.1)
C. lusitaniae	8.5 ± 2.12	0.028 ± 0.012 (−99.7)	20.1 ± 5.9 (+136.5)	131 ± 39 (+1,441.2)
C. parapsilosis	4.0 ± 1.4	0.129 ± 0.026 (−96.8)	12.8 ± 0.28 (+220.0)	13.2 ± 2.9 (+230)
C. kefyr	25.0 ± 2.4	0.092 ± 0.025 (−99.6)	60.5 ± 20.5 (+142)	66 ± 19 (+164)
C. stellatoidea	12.5 ± 4.2	0.00005 (−100)	84.0 ± 21.4 (+572)	108 ± 43 (+764)
C. tropicalis	11.7 ± 2.5	0.0067 (−99.9)	137 ± 65 (+1,070.9)	150 ± 37 (+1,182.1)
C. glabrata	7.9 ± 0	0.00005 (−100)	152 ± 30 (+2,071.4)	46.5 ± 23.3 (+564.3)

[a] Results shown are from a typical experiment. Each value represents the mean of two independent determinations. T_0 and T_{24} denote the times immediately prior to and 24 h after addition of the antifungal agent, respectively.
[b] Percent change is the decrease or increase from the original inoculum (T_0). AMB, amphotericin B; ITZ, itraconazole; VCZ, voriconazole. All drugs were used at 5 μg/ml.

In a separate study investigators (Lass-Florl et al., 2001, AAC 45:124-128) assessed the cidal activity of voriconazole against the filamentous stage of growth for various species of *Aspergillus*. Utilizing a FUN-1 stain, which turns red when taken up by actively metabolizing fungal cells, investigators were able to differentiate live and dead hyphal elements. Table 4 shows the MFC values for voriconazole and itraconazole against the hyphal phase of growth. The MFCs against the hyphal phase of growth are comparable for voriconazole and itraconazole. These MFC values against the hyphal phase are 8-16

fold higher than the MFCs obtained when *Aspergillus* conidia were used to calculate the MFC. While the MFCs for voriconazole and itraconazole are similar for the majority of the isolates tested, it should be noted that some *Aspergillus* isolates had higher MFC for one drug or the other suggesting different mechanisms of resistance may be specific to voriconazole or itraconazole. However, additional data are needed to confirm this observation.

TABLE 11 Susceptibility of *Aspergillus* hyphae to voriconazole and itraconazole

Aspergillus sp.[a] (no. of isolates tested)	Voriconazole MFC range (μg/ml) at 48 h	Voriconazole staining pattern(s)[b]	Itraconazole MFC range (μg/ml) at 48 h	Itraconazole staining pattern(s)
A. flavus (5)	8->16	VID	4-4	ID
	2-2	D	2-2	D
	2-4	D	2-2	D
	8->16	VD	4-4	D
	2-4	D	4-8	D
A. terreus (5)	2-4	D	4-4	D
	2-4	ID	4-4	ID
	16	VD	2-2	D
	2-4	D	8->16	VD
	2-2	D	4-4	D
A. niger (5)	8-16	ID	8->16	VD
	4-4	D	4-4	D
	2-2	D	8->16	VD
	2-2	D	2-2	D
	2-2	D	2-2	D
A. fumigatus (5)	2-2	D	2-2	D
	2-4	ID	2-4	ID
	8->16	VID	4-4	ID
	4-4	D	2-2	D
	2-2	D	2-4	D
Itraconazole-resistant *A. fumigatus* (1)	2-2	D	>16	VID

[a] Each isolate was tested three times.
[b] V, Viable hyphae represented by green fluorescent hyphae with red vacuolar structures; I, impaired hyphae represented by green fluorescent hyphae and lack of red fluorescent vacuolar structures; D, dead hyphae represented by green-yellow fluorescence without any vacuolar structures. The predominant patterns are underlined.

In an in vitro study conducted by Ruhnke et. al.(1997, AAC 41:575-7) voriconazole susceptibility testing was performed against fluconazole susceptible and resistant isolates of *C. albicans* obtained from HIV positive patients with oropharyngeal candidiasis. The NCCLS M27-T method was used where the final concentrations of voriconazole and fluconazole ranged from 0.048 to >100 μg/ml. Susceptibility results were read after 48 hours incubation. The investigators in this study used different dilution factors than those proposed by the NCCLS and chose a breakpoint of ≥25 μg/ml to define resistance. This FDA reviewer chose to evaluate the data using breakpoints closer to those proposed by the NCCLS (MIC of ≥64 μg/ml). For the purpose of this review fluconazole MICs of ≤ 12.5 μg/ml and ≥100 μg/ml were employed to signify fluconazole susceptible and resistant *C. albicans* isolates, respectively.

Using these criteria 24/27 (90%) isolates with a fluconazole MIC ≥ 100 μg/ml had a voriconazole MIC ≥ 0.39 μg/ml. In this study there were 56 *C. albicans* isolates with fluconazole MICs < 12.5 μg/ml. The NCCLS defines fluconazole susceptible *Candida*

when the MIC is $\leq$ 8.0 µg/ml. Of the 56 fluconazole susceptible isolates 44 (79%) and 53 (95%) had voriconazole MICs of $\leq$ 0.09 µg/ml and $\leq$ 0.19 µg/ml, respectively. The MIC results as shown in Figure 2 indicate that there is good correlation between fluconazole susceptible and resistant *C. albicans* isolates and voriconazole MICs of $\leq$ 0.09 µg/ml and $\geq$ 0.39 µg/ml, respectively. However, it is unclear what voriconazole concentrations would be used to define the dose dependant susceptible (DDS) range.

Figure 2

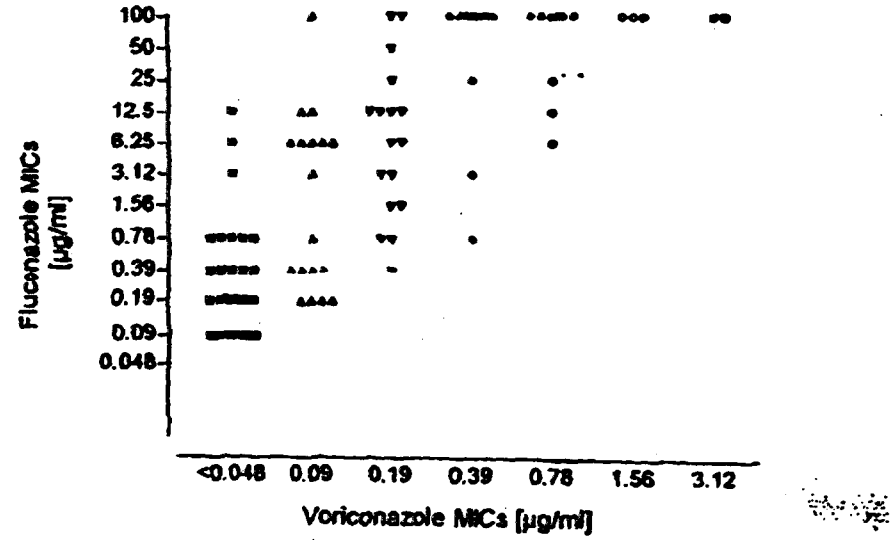


FIG. 2. Scatter plots of in vitro susceptibilities to voriconazole versus fluconazole for 105 *C. albicans* isolates tested by M27-T-micro (9) (A) and HR-micro (15) (B). Each datum point represents one experiment. (A) ◆, n = 40; ▲, n = 18; ▼, n = 17; ◆, n = 14; ●, n = 11; ○, n = 3; ■, n = 2. (B) ◆, n = 62; ▲, n = 2; ▼, n = 15; ◆, n = 7; ●, n = 15; □, n = 1; ○, n = 3; ■, n = 1.

The in vitro activity of voriconazole against *Aspergillus* species was studied by Murphy et. al. (Antimicrob. Agents Chemother. 1997, 41:696-698). Using a modified NCCLS M27-A method voriconazole, itraconazole and amphotericin B MICs were determined for *A. fumigatus*, *A. flavus* and *A. niger* isolates. The susceptibility results show that the MIC_{90} for voriconazole were within a 2-fold dilution of those found for itraconazole for all 3 *Aspergillus* species tested (See table 5). Voriconazole and itraconazole MIC_{90} for the three *Aspergillus* species were 0.25 to 0.5 µg/ml, indicating comparable in vitro activity.

Table 5

In Vitro Susceptibility Results of Three *Aspergillus* Species

Results of tests of in vitro susceptibilities of *Aspergillus* spp. to voriconazole, itraconazole, and amphotericin B

Drug	MIC (μg/ml)*								
	A. fumigatus (*n* = 21)			*A. flavus* (*n* = 10)			*A. niger* (*n* = 10)		
	Range								
Voriconazole	<0.03–0.5	0.25	0.03	0.25–0.5	0.5	0.5	0.25–1.0	0.5	0.25
Itraconazole	<0.03–1.0	0.5	0.06	0.125–0.25	0.25	0.25	0.5–2.0	1.0	1.0
Amphotericin B	0.5–2.0	2.0	1.0	1.0–4.0	4.0	2.0	0.5–1.0	1.0	0.5

* 90% and 50%, MICs at which 90 and 50% of isolates, respectively, are inhibited.

Radford and colleagues studied the in vitro activity of voriconazole against less common moulds (Antimicrob. Agents Chemother. 1997, 41: 841-843). The information in table 6 was taken from a more complex table published in the article. MICs were determined for the conidial form of the moulds grown on High-Resolution (HR) medium. It should be noted that this is a different susceptibility testing method than the NCCLS M38-P and MIC values can not be compared to those obtained using the NCCLS M38-P method as comparability tests were not performed.

Table 6

In vitro Activities of Voriconazole and Itraconazole

Organism (# of isolates)	**Voriconazole** MIC range (μg/ml)	**Itraconazole** MIC range (μg/ml)
Fusarium oxysporum (3)	0.5-2.0	0.25->64
Fusarium solani(4)	1.0-4.0	>64
Scedosporium apiospermum (6)	0.12-0.5	0.12->64
Scedosporium prolificans (5)	4.0	>64

Clancy studied the inhibitory and cidal activity of voriconazole against *Aspergillus* and *Fusarium* species (J. Clin. Microbiol. Infect. Dis. 1998 17:573-575). The NCCLS macrodilution method was employed to determine voriconazole and amphotericin B MICs and MLCs against 20 *A. fumigatus*, 9 *A. flavus*, 11 *F. solani*, 6 *F. proliferatum*, 5 *F. oxysporum* and 3 *F. moniliforme*. Tables 7 and 8 show the MIC and MLC patterns for the various species tested. The voriconazole MICs for the *Aspergillus* species were lower than those for the *Fusarium* species. In addition, the MLCs for the *Fusarium* species were >16 µg/ml suggesting that infections produced by these moulds are more difficult to treat than those produced by *Aspergillus flavus* or *A. fumigatus* where the MLCs ranged form 0.25- 8 µg/ml.

Table 7

Distribution of Voriconazole and Amphotericin B MICs against *Aspergillus* and *Fusarium* isolates

Organism (no.)	Voriconazole MIC (µg/ml)					Amphotericin B MIC (µg/ml)			
	0.125	0.25	0.5	1	2	0.25	0.5	1	2
All *Aspergillus* spp. (29)	7	5	13	3	1	2	14	13	0
A. fumigatus (20)	7	4	7	2	0	2	11	7	0
A. flavus (9)	0	1	6	1	1	0	3	6	0
All *Fusarium* spp. (25)	0	2	1	11	11	0	5	14	6
F. solani (11)	0	1	0	5	5	0	3	7	1
F. proliferatum (6)	0	0	0	3	3	0	0	2	4
F. oxysporum (5)	0	1	0	2	2	0	2	2	1
F. moniliforme (3)	0	0	1	1	1	0	0	3	0

Table 8

Minimum Lethal Concentrations of Voriconazole and Amphotericin B against *Aspergillus* and *Fusarium* Isolates

Organism (no.)	Voriconazole MLC (µg/ml)							Amphotericin MLC (µg/ml)					
	0.25	0.5	1	2	4	8	≥16	0.25	0.5	1	2	4	8
Aspergillus spp. (29)	1	2	11	7	3	5	0	2	12	13	2	0	0
Fusarium spp. (25)	0	0	0	1	2	2	20	0	2	13	8	1	1

In house studies were conducted by Pfizer to determine the in vitro activity of voriconazole when used in combination with amphotericin B or 5-fluoro-cytosine (5FC). were set up in a checkerboard pattern where varying concentrations of voriconazole or fluconazole were combined with amphotericin B or 5FC. The various drug combinations were evaluated against a single isolate of *C. albicans* and *A. fumigatus*. The test results showed that the addition of either amphotericin B or 5FC had

no effect (i.e. additive, synergistic or antagonistic) on the activity voriconazole or fluconazole against *C. albicans* when used in combination (data not shown). When tested against *A. fumigatus* the in vitro activity of fluconazole was not altered by the addition of amphotericin B or 5FC. However, one could not discern if amphotericin B or 5FC were antagonistic when used in combination with fluconazole as the *Aspergillus* isolate evaluated was inherently resistant to fluconazole with an MIC of >100 µg/ml and higher concentrations of fluconazole were not studied. It was observed that when tested against *A. fumigatus* voriconazole MICs rose by two fold in the presence of increasing concentrations of amphotericin B and 4 fold when used in combination with increasing concentrations of 5FC. While these increases are not great, they do suggest that the activity of voriconazole against *A. fumigatus* is reduced when used in combination with amphotericin B or 5FC. This drug combination information is interesting; however, it should be noted that the data were obtained from only one isolate of each fungal species. Additional in vitro and in vivo combination drug studies must be performed to adequately characterize the voriconazole when used in combination with other antifungal agents.

In Vivo Studies:

The in vivo activity of oral voriconazole was studied by the sponsor utilizing normal and immuno-compromised guinea pigs infected with *A. fumigatus* and *C. albicans* isolates. In one study normal guinea pigs with established systemic *A. fumigatus* infection (treatment initiated 48 hours post infection) were treated with various antifungal agents. Oral voriconazole and itraconazole at 5 and 10 mg/kg b.i.d. produced the greatest reduction in the fungal burden in kidney tissue. At the 1 mg/kg dose voriconazole and itraconazole reduced the fungal burden by 1.3 and 1.8 $\log_{10}$, respectively (See table 9).

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Table 9

Activity of UK-109,496, itraconazole, fluconazole and amphotericin B against systemic aspergillosis in immune normal guinea pigs

Compound	Dose (mg/kg)	Survivors	Cures	*Aspergillus* content of kidney (CFU/g homogenate)
UK-109,496	10	15/15	15/15	0.00***
	5	15/15	12/15	0.10***
	1	8/15	4/15	1.90 ± 1.47
Itraconazole	10	15/15	9/15	0.31***
	5	13/15	7/15	0.39***
	1	8/15	2/15	1.39 ± 1.29
Fluconazole	50	12/15	1/15	2.60 ± 1.05
Amphotericin B	4	3/5	0/5	2.95 ± 0.75
Controls	0	0/13	0/13	3.21 ± 0.31

Animals were dosed orally b.i.d. for 5 days except for amphotericin B which was administered i.p. o.d. for 5 days, commencing 1h post-infection. The vehicle was 0.5ml polyethylene glycol 200
Kidneys were harvested 16h after the last dose

Values are means, ± S.D., of the results obtained from 3 separate experiments with groups of 5 animals, except where cures were observed, when an average value only is given

CFU/g = $\log_{10}$ colony-forming units per g of kidney homogenate

Significance of difference from vehicle group (Student's independent t-test) ***P <0.001

In neutropenic guinea pigs antifungal therapy was started 1-hour post infection, mimicking a new non-established systemic infection model. After 4 days of therapy oral voriconazole at 5 mg/kg b.i.d. reduced the fungal burden in the liver of animals 2 $\log_{10}$ greater than the same dose of itraconazole (See table 10).

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Table 10

Activity of UK-109,496, itraconazole, fluconazole and amphotericin B against systemic aspergillosis in cyclophosphamide- and dexamethasone-immunocompromised guinea pigs

Compound	Dose (mg/kg)	Survivors	Cures	*Aspergillus* content of liver (CFU/g tissue)
UK-109,496	10	27/28	28/28	0.00***
	5	28/28	14/28	0.32***
	2.5	8/10	0/10	1.70 ± 0.10**
	1	4/28	0/28	3.26 ± 0.44
Itraconazole	10	27/27	4/27	0.66 ± 0.58***
	5	21/28	2/28	2.39 ± 0.85
	1	3/25	0/25	3.30 ± 0.39
Fluconazole	20	3/17	0/17	3.21 ± 0.32
Amphotericin B	4	3/9	0/9	3.26 ± 0.64
Vehicle	0	1/25	0/25	3.19 ± 0.40

Animals were immunocompromised with 2mg/kg p.o. dexamethasone daily, starting 4 days prior to infection plus two 100mg/kg i.p. doses of cyclophosphamide 4 days and 1 day prior to infection

Animals were dosed orally b.i.d. for 4 days except for amphotericin B which was administered i.p. o.d. for 4 days, commencing 4h post-infection. The vehicle was 0.5ml polyethylene glycol 200

Livers were harvested 16h after last dose

Values are means, ± S.D. , of the results obtained from 3 separate experiments with groups of 8 to 8 animals, except when cures were observed, when an average value only is given

CFU/g = $\log_{10}$ colony-forming units per g of liver

Significance of difference from vehicle group (Student's independent t-test) ** P <0.01 *** P <0.001

When an *A. fumigatus* isolate demonstrating reduced activity to itraconazole (itraconazole MIC 3.1 µg/ml) was used to produce a systemic infection in immunocompromised guinea pigs, voriconazole at 5 mg/kg and 10 mg/kg significantly reduced the fungal burden in liver tissue. These data suggest that voriconazole retained its activity against an *Aspergillus* strain that exhibited a particular mechanism of resistance to itraconazole (See table 11).

Table 11

Compilation of experiments UK109496/DI/20/97 (lab book 4832 page 116) and UK109496/DI/21/97 (lab book 4832 page 126), azole-resistant strain H98.53

Treatment	mg/kg	Survivors	Cures (zero count)	n	Mean $\log_{10}$CFU/g ± SD	Log_{10} advantage over vehicle
Voriconazole b.i.d. x4	10	9/12	11/12	12	<0.04*	2.95
	5	9/12	11/12	12	<0.05*	2.84
	1	10/12	0/12	12	1.68 ± 0.58**	1.21
Itraconazole b.i.d. x4	20	8/12	0/12	12	2.21 ± 0.47**	0.68
	10	11/12	0/12	12	2.21 ± 0.43**	0.68
	5	10/11	0/11	11	2.4 ± 0.50 NS	0.49
	1	6/6	0/6	6	2.45 ± 0.14 NS	0.44
Vehicle	0	12/12	0/12	12	2.71 ± 0.22	-

*p<0.001, **p<0.01, NS = not significant

In vivo studies were also conducted against pulmonary *A. fumigatus* infections in immunocompromised guinea pigs. Animals were intratracheally infected with 5 $x10^5$ spores. Daily treatment started 24 hours post infection and went through day 7. Lung tissue was harvested 16 hours post therapy and cultured for fungi. In this acute pulmonary infection model, where few untreated infected control animals died, oral voriconazole administered at 8, 4 and 2 mg/kg b.i.d. produced the greatest drop in fungal burden in lung tissue. Itraconazole at 8 and 4 mg/kg b.i.d produced a 0.8 log_{10} drop (See Table 12).

Table 12

Activity of UK-109,496, Itraconazole and fluconazole against pulmonary aspergillosis in cortisone acetate-immunocompromised guinea pigs

Compound	Dose (mg/kg)	Survivors	Cures	*Aspergillus* content of lung (CFU/g tissue)
UK-109,496	8	22/22	11/22	0.21*** ###
	4	18/20	6/20	0.46*** ###
	2	14/14	2/14	0.89 ± 0.91
Itraconazole	8	18/18	2/18	1.24 ± 1.05
	4	17/18	2/18	1.15 ± 0.95
Fluconazole	50	17/17	3/17	1.02 ± 0.98
Vehicle	0	16/19	0/19	2.01 ± 1.21

Animals were immunocompromised with six 100mg/kg s.c. doses of cortisone acetate 7, 4 and 2 days prior to infection, on the day of infection, and 3 and 5 days post-infection

Animals were dosed orally b.i.d. for 7 days, commencing 24h post-infection. The vehicle was 0.5ml polyethylene glycol 200

Lungs were harvested 16h after last dose

Values are means, ± S.D., of results from 3 separate experiments with groups of 6 to 8 animals, except where cures were observed, when an average value only is given

CFU/g = log_{10} colony-forming units per g of lung.

Significance of difference from vehicle group (Student's independent t-test) *** P <0.001

Significance of difference from itraconazole group (Student's independent t-test) ### P <0.001

Pfizer conducted one in house experiment assessing the activity of voriconazole in immune competent guinea pigs with systemic *Scedosporium apiospermum* infections. For this isolate voriconazole and itraconazole MICs were 0.39 µg/ml. Animals were infected intravenously with 4 x 10^6 conidia. Animals were treated for 5 (part A) or 10 (part B) days starting 1 hour post-infection. Sixteen hours post therapy animals were sacrificed and kidney, lung, liver, spleen and brain tissues removed and cultured for fungi. At day 5 of treatment voriconazole had produced a significant drop in fungal burden in brain tissue. An interim assessment of itraconazole's activity was not determined on day 5. After 10 days of therapy voriconazole at 5 and 10 mg/kg BID dosing produced 100% survival with a significant drop in fungal burden in all tissues. Itraconazole at 10 mg/kg BID dosing produced a significant drop in fungal burden in only brain and spleen tissue

(See table 13). From this single animal infection study it appears that voriconazole at high doses has activity against *Scedosporium apiospermum* infections in normal guinea pigs. However, additional in vivo studies need to be performed to fully characterize the activity of voriconazole against this fungal pathogen.

Table 13

Compilation of experiments UK/109406/DI/10/98 and UK/109406/DI/003/99. [Part B – 10-day Treatment]

Treatment	mg/kg	Survivors	n	a) Mean log_{10}CFU/g ± SD				
				Brain	Lung	Liver	Kidney	Spleen
Voriconazole b.i.d.	10	11/11	11	<0.08***	0.65 ± 0.27***	1.44 ± 0.19***	0.33 ± 0.28***	2.62 ± 0.29***
	5	11/11	11	<0.08***	0.92 ± 0.43**	1.58 ± 0.34***	0.50 ± 0.39***	2.88 ± 0.44***
	2.5	3/11	11	<0.07***	1.71 ± 0.46NS	2.68 ± 0.58NS	1.32 ± 0.40**	3.64 ± 0.52***
Itraconazole b.i.d.	10	1/10	10	1.02 ± 0.78***	1.83 ± 0.52*	2.83 ± 0.87NS	1.78 ± 0.38*	4.22 ± 0.46***
Vehicle	0	0/11	11	2.89 ± 0.51	2.23 ± 0.74	2.96 ± 0.24	2.11 ± 0.26	5.01 ± 0.44
Treatment	mg/kg			b) Log_{10} advantage over vehicle (curve)				
				Brain	Lung	Liver	Kidney	Spleen
Voriconazole b.i.d.	10			>2.61 (11/11)	1.58 (0/11)	1.52 (0/11)	1.78 (0/11)	2.40 (0/11)
	5			>2.61 (10/11)	1.31 (0/11)	1.38 (0/11)	1.61 (0/11)	2.13 (0/11)
	2.5			>2.62 (4/11)	0.47 (0/11)	0.28 (0/11)	0.79 (0/11)	1.37 (0/11)
Itraconazole b.i.d.	10			1.67 (0/10)	0.80 (0/10)	0.13 (0/10)	0.33 (0/10)	0.79 (0/10)

Significance of differences from vehicle treatment: * p<0.05 ** p<0.01 ***p<0.001 NS not significant

An azole susceptible isolate of *C. albicans* was used to produce a non-established systemic infection in normal guinea pigs. Oral voriconazole, fluconazole and itraconazole were administered b.i.d. for 5 days starting 1-hour post infection. Sixteen hours after the last dose animals were sacrificed and kidneys were removed for fungal culture. In kidney tissue a 4 log_{10} drop in fungal burden was observed in animals administered 1 mg/kg b.i.d. fluconazole, 5 mg/kg itraconazole and 5 mg/kg voriconazole (See table 14).

Table 14

Activity of UK-109,496, Itraconazole, fluconazole and amphotericin B against systemic candidosis in immune normal guinea pigs

Compound	Dose (mg/kg)	Survivors	*Candida* content of kidney (CFU/g tissue)
UK-109,496	5	17/17	0.23 ± 0.21***
	1	14/14	2.32 ± 0.51*
	0.1	10/14	3.54 ± 1.07
Itraconazole	5	17/17	0.11 ± 0.46***
	1	14/14	2.67 ± 0.75*
	0.1	9/17	4.19 ± 0.99
Fluconazole	5	17/17	0.03 ± 0.56***
	1	17/17	0.60 ± 0.61***
	0.1	12/14	3.40 ± 0.96
Amphotericin B	2	7/11	2.91 ± 0.80
Controls	0	13/18	4.41 ± 0.74

Animals were dosed orally b.i.d. for 5 days except for amphotericin B which was administered i.p. o.d. for 5 days, commencing 1h post-infection. The vehicle was 0.5ml polyethylene glycol 200

Kidneys were harvested 16h after the last dose

Values are means, ± S.D., of the results obtained from 3 separate experiments with groups of 5 to 6 animals

CFU/g = $\log_{10}$ colony-forming units per g of kidney

Significance of difference from vehicle group (Student's independent t-test) * $P < 0.05$ *** $P < 0.001$

In a second study conducted by Pfizer immunocompromised guinea pigs were intravenously infected with a fluconazole susceptible *C. albicans* isolate. Oral b.i.d. dosing was initiated 1 hour post infection and continued for 4 days. The 5 mg/kg b.i.d. dose of itraconazole and the 1 and 5 mg/kg b.i.d. doses of fluconazole produced at least a 5 $\log_{10}$ drop in fungal burden in the kidney tissue of infected animals. Voriconazole at 5 mg/kg reduced the fungal burden by 4.5 $\log_{10}$ (See table 15). Survival and reduction in fungal burden in kidney in all treatment arms at all doses were not as great as those seen in the same study conducted in normal guinea pigs. These data confirm that the immune status of the host has a direct impact on the clinical outcome of animals with fungal infections.

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Table 15

Activity of UK-109,496, itraconazole, fluconazole and amphotericin B against systemic candidosis (*C.albicans*) in cyclophosphamide- and dexamethasone-immunocompromised guinea pigs

Compound	Dose (mg/kg)	Survivors	*Candida* content of kidney (CFU/g tissue)
UK-109,496	5	15/24	1.44 ± 0.94***
	1	6/24	4.17 ± 0.67*
	0.1	0/17	5.61 ± 0.84
Itraconazole	5	16/24	0.79 ± 1.10***
	1	14/24	3.28 ± 1.02*
	0.1	0/16	5.83 ± 0.40
Fluconazole	5	15/21	0.66 ± 0.78***
	1	17/23	1.09 ± 0.67***
	0.1	0/17	5.61 ± 0.80
Amphotericin B	2	1/12	5.07 ± 1.26
Vehicle	0	0/16	6.09 ± 0.42

Animals were immunocompromised with 2mg/kg p.o. dexamethasone daily, starting 6 days prior to infection plus three 100mg/kg i.p. doses of cyclophosphamide on days 6, 3 and 1 prior to infection.

Animals were dosed orally b.i.d. for 5 days, commencing 1h post infection except for amphotericin B which was administered i.p. on the day of infection and on days 2 and 4 post-infection. The vehicle was 0.5% polyethylene glycol 200

Kidneys were harvested 16h after last dose

Values are means, ± S.D., of the results obtained from 3 separate experiments with groups of 6 to 8 animals

CFU/g = $\log_{10}$ colony-forming units per g of kidney.

Significance of difference from vehicle group (Student's independent t-test) * P <0.05 *** P <0.001

Voriconazole activity was assessed in immunocompromised guinea pigs intravenously infected with a fluconazole resistant strain of *C. albicans*. Animals were infected with 5 x 10^5 CFU/ml. Treatment commenced 1 hour post infection and continued for 4 days. Kidneys were harvested 16 hours after the last dose of drug and cultured for fungi. Oral b.i.d. doses of voriconazole at 10 mg/kg and 20 mg/kg were effective in reducing the fungal burden in the kidneys by approximately 3 $\log_{10}$ (See table 16). Itraconazole and fluconazole at 20 mg/kg were not effective in this animal infection model. These data suggest that the antifungal activity of voriconazole is not affected by this particular azole mechanism of resistance exhibited by this particular *C. albicans* strain. However, it should be noted that the exact mechanism of resistance was not identified or specified in the article.

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Table 16

Activity of UK-109,496, itraconazole and fluconazole against azole-resistant systemic candidosis (*C. albicans*) in cyclophosphamide- and dexamethasone-immunocompromised guinea pigs

Compound	Dose (mg/kg)	Survivors	*Candida* content of kidney (CFU/g tissue)
UK-109,496	10	14/16	0.59 ± 0.12***, a, b
	5	14/18	2.54 ± 1.12
	1	11/15	3.91 ± 0.78
Itraconazole	10	13/16	4.00 ± 0.73
	5	11/15	3.93 ± 0.81
	1	5/6	3.76 ± 0.60
Fluconazole	20	9/15	3.99 ± 0.73
Vehicle	0	10/15	3.86 ± 0.77

Animals were immunocompromised with 2mg/kg p.o. dexamethasone daily, starting 4 days prior to infection plus two 100mg/kg i.p. doses of cyclophosphamide 4 days and 1 day prior to infection

Animals were dosed orally b.i.d. for 4 days, commencing 1h post-infection. The vehicle was 0.5ml polyethylene glycol 200

Kidneys were harvested 16h after last dose

Values are means, ± S.D., of the results obtained from 3 separate experiments with groups of 5 to 6 animals

CFU/g = $\log_{10}$ colony-forming units per g of kidney.

Significance of difference from vehicle group (Student's independent t-test) *** P <0.001
Significance of difference from itraconazole group (Student's independent t-test) a P <0.001
Significance of difference from fluconazole group (Student's independent t-test) b P <0.001

Immunocompromised guinea pigs were intravenously infected with *C. krusei* and *C. glabrata*, respectively. All animals were treated for 7 days starting 1-hour post infection. Animals were sacrificed 16 hours post therapy and kidneys removed for fungal culture. In these models oral voriconazole at 10 mg/kg and 20 mg/kg reduced the fungal burden in the kidney by approximately 2 $\log_{10}$. In the *C. krusei* model itraconazole and fluconazole at 20 mg/kg reduced the fungal burden in tissue by 0.4 to 0.6 $\log_{10}$. In the *C. glabrata* model, itraconazole at 20 mg/kg was not effective, however, fluconazole at 20 mg/kg produced a 2-$\log_{10}$ drop in kidney tissue (See tables 17 & 18). The activity of voriconazole was not determined in other tissues such as lung or liver.

Table 17

Activity of UK-109,496, itraconazole, fluconazole and amphotericin B against systemic Candida glabrata infection in cyclophosphamide- and dexamethasone-immunocompromised guinea pigs

Compound	Dose (mg/kg)	Survivors	*Candida* content of kidney (CFU/g tissue)
UK-109,496	10	8/8	1.63 ± 0.68*** #
	5	8/8	3.26 ± 0.83
	1	8/8	4.18 ± 0.23
Itraconazole	10	8/8	3.72 ± 0.80
	5	8/8	3.98 ± 0.60
	1	8/8	3.75 ± 1.02
Fluconazole	20	8/8	2.06 ± 1.30*
Amphotericin B	1	8/8	2.11 ± 1.41
Vehicle	0	9/9	4.04 ± 0.51

Animals were immunocompromised with 2mg/kg p.o. dexamethasone daily, starting 4 days prior to infection plus two 100mg/kg i.p. doses of cyclophosphamide 4 days and 1 day prior to infection

Animals were dosed orally b.i.d. for 7 days except for amphotericin B which was administered i.p. o.d. on alternate days, commencing 1h post-infection. The vehicle was 0.5ml polyethylene glycol 200

Kidneys were harvested 15h after the last dose

Values are means, ± S.D., of the results obtained from one experiment with groups of 8 or 9 animals

CFU/g = $\log_{10}$ colony-forming units per g of kidney

Significance of difference from vehicle group (Student's independent t-test): * P <0.05; *** P <0.001

Significance of difference from itraconazole group (Student's independent t-test): # P <0.05

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Table 18

Activity of UK-109,496, itraconazole, fluconazole and amphotericin B against systemic *Candida krusei* infection in cyclophosphamide- and dexamethasone-immunocompromised guinea pigs

Compound	Dose (mg/kg)	Survivors	*Candida* content of kidney (CFU/g tissue)
UK-109,496	10	8/8	0.10 ± 0.05***a,b
	5	8/8	0.66 ± 0.21***a,b
	1	8/8	2.03 ± 0.62
Itraconazole	10	8/8	1.08 ± 1.83
	5	8/8	2.11 ± 1.01
	1	8/8	2.01 ± 1.13
Fluconazole	20	8/8	1.37 ± 0.97
Amphotericin B	1	8/8	1.23 ± 0.42
Vehicle	0	9/9	1.93 ± 0.80

Animals were immunocompromised with 2 mg/kg p.o. dexamethasone daily, starting 4 days prior to infection plus two 100 mg/kg i.p. doses of cyclophosphamide 4 days and 1 day prior to infection

Animals were dosed orally b.i.d. for 7 days, except for amphotericin B which was administered i.p. o.d. on alternate days for 7 days, commencing 1h post-infection. The vehicle was 0.5ml polyethylene glycol 200

Kidneys were harvested 16 h after the last dose

Values are means, ± S.D., of the results obtained from one experiment with groups of 8 or 9 animals

CFU/g = $\log_{10}$ colony-forming units per g of kidney

Significance of difference from vehicle group (Student's independent t-test) *** P <0.001

Significance of difference from itraconazole group (Student's independent t-test) a P <0.001

Significance of difference from fluconazole group (Student's independent t-test) b P <0.001

Ghannoum et. al., (J. Chemother.1999, 11:1-34-39) studied the activity of voriconazole, amphotericin B and fluconazole in neutropenic guinea pigs newly infected with *C. krusei*. One-hour post infection neutropenic guinea pigs were administered drug for 7 days. Twenty-four hours post therapy animals were sacrificed and brain, liver and kidney tissues were harvested and cultured for fungi. The culture results found in table show that voriconazole at 5 and 10 mg/kg produced a greater reduction in fungal burden in all three tissues versus fluconazole or amphotericin B (See table 19).

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Table 19

- *Activity of voriconazole (VOR), fluconazole (FLU) and amphotericin B (AmB) against C. krusei infection in neutropenic guinea pigs.*

Group	Brain	Liver	Kidney
Control (n=14)	3.3±0.3	2.0±1.4	1.8±0.8
AmB (n=9)	3.3±0.1	1.6±0.9	1.4±0.2
FLU (n=8)	2.9±0.4	1.7±0.4	1.4±0.2
VOR (n=8) (5 mg/kg bid)	1.2±1.7*	0.72±0.5*	0.27±0.1*
VOR (n=8) (10 mg/kg/bid)	1.1±0.7*	0.5±0.1*	0.7±0.4*

Data presented as mean Log_{10} CFU±SD of *C. krusei* /g of tissue
* $P < 0.01$ compared with control.

Animal infection studies were not performed evaluating the activity of voriconazole in combination with other antifungal agents. At this time it is unknown how the in vivo activity of voriconazole would be affected when used in combination with other antifungal agents.

Clinical Microbiology:

Treatment of Invasive Aspergillosis

A. Clinical Microbiology Review of Controlled Aspergillus studies 150-307 and 150-602:

Clinical trial design

Please refer to the Medical Officer's review for a detailed description of the clinical trial design.

Clinical Microbiology Results:

Clinical trials 150-307 and 150-602 were designed such that subjects with definite or probable acute invasive aspergillosis, based on disease criteria defined by the Mycoses Study Group (MSG), were eligible to receive either amphotericin B or voriconazole. The immunosuppressive state of the subjects had to be due to one of the following: allogenic or autologous bone marrow or peripheral stem cell transplant, haematologic malignancies, aplastic anemia and myelodysplastic syndrome, solid organ transplant, HIV, solid organ malignancy or after high dose prolonged corticosteroid or other immunosuppressive therapy. Because the design of the two clinical trials was essentially identical (307 conducted in Europe and 602 conducted in N. and S. America) the data from both studies were pooled and analyzed as one data set. As a consequence, the microbiologic discussion will pertain to the pooled database unless otherwise specified.

As per the design of the clinical trials, clinical and microbiologic efficacy were assessed at weeks 6, 12, the end of randomized therapy (EORT) and at week 16. However, the primary efficacy analysis was performed at week 12. For the purpose of this review the global response for each subject as determined by the data review committee (DRC) at week 12 was used to define the modified-intent-to-treat (MITT) population. Subjects were categorized as having a satisfactory or unsatisfactory response. Patients with satisfactory responses included those subjects that had a complete or partial response at week 12. Subjects categorized as unsatisfactory had at week 12 a clinical response of either, stable, indeterminate or failure.

To characterize the microbiologic activity of voriconazole against *Aspergillus* isolates, only subjects from the MITT population with fungal cultures positive for *Aspergillus* were looked at in greater detail. To be considered a potential pathogen, an *Aspergillus* isolate had to be isolated from a sterile site or from sputum with other clinical signs and symptoms suggestive of an *Aspergillus* infection. In the combined data set there were 77/144 (53%) subjects in the voriconazole arm and 68/133 (51%) subjects in the amphotericin B arm that met these criteria. Table 20 shows the number of subjects in each treatment arm that were in the various evaluable patient populations.

Table 20

Patient Populations Evaluated in Aspergillus Studies 307 and 602

Evaluation Groups	Voriconazole Arm	Amphotericin B
Patients entered and treated	196	185
MITT population	144	133
MITT with Aspergillus Positive cultures	77	68

MITT= modified intent-to-treat population

Culture proven cases of Aspergillosis were classified according to whether a single fungal species or multiple fungal species were recovered from clinically relevant sites (i.e. respiratory and/or sterile sites). To be included in this assessment MITT subjects had to have had at least one *Aspergillus* species that was recovered from a clinically relevant site. Table 21 shows the number of patients in each treatment arm that had single or multiple fungal species isolated from clinically relevant body sites. In the voriconazole arm 50/77 (65%) and 41/68 (60%) of the MITT patients had documented fungal infections due to a single *Aspergillus* isolate. Multiple moulds were recovered from relevant clinical sites in 3/77 (4%) and 7/68 (6%) of the patients in the voriconazole and amphotericin B arms, respectively. There was a greater incidence of MITT patients with *Aspergillus* and yeast recovered from clinically relevant sites in both treatment arms, 22/77 (29%) in the voriconazole arm and 18/68 (26%) in the amphotericin B arm. Two subjects in each treatment arm had multiple yeasts and or moulds recovered from clinically relevant sites.

Table 21

Distribution of Fungal Species Recovered from Patients in the MITT Population From Aspergillus studies 307/602

Fungal Strains Recovered	Voriconazole	Amphotericin B
Single Aspergillus species	50	41
2 Aspergillus species	1	5
Aspergillus +Non-Asp. Mould	1*	0
Aspergillus + ≥2 moulds	1	2 (1*)
Aspergillus +1 yeast	20	13
Aspergillus +≥2 yeast	2	5
Aspergillus + yeasts and moulds	2*	2
Total	77	68

*= patients with a Zygomycete mould; Asp.= Aspergillus

To further compare the efficacy of voriconazole to amphotericin B in the treatment of *Aspergillus* infections, the data were analyzed to determine the satisfactory and unsatisfactory response rates observed in the two treatment arms against the various *Aspergillus* species. The ability to compare the efficacy of voriconazole to amphotericin B against the various *Aspergillus* species was confounded by two factors; 1) the number of subjects that had documented infections due to *A. fumigatus* versus non-fumigatus *Aspergillus* species and 2) the number of subjects that had multiple fungi recovered from clinically relevant sites.

Infections due to *A. fumigatus* versus non-fumigatus species of *Aspergillus* were first studied in detail. In the voriconazole arm there were 50/77(65%) subjects who had infections due to a single species of *Aspergillus* of which 43/50 (86%) were *A. fumigatus* (See table 22). Of the 43 voriconazole patients with culture proven infections due to *Aspergillus fumigatus* alone 22(51%) had a satisfactory response at the end of study week 12. Only 1 patient deteriorated between weeks 12 and week 16. The remaining 7 subjects (14%) in the voriconazole arm were infected with a single species of a non-fumigatus *Aspergillus* which included 3 *A. flavus*, 1 *A. terreus*, 2 *A. niger* and 1 *Aspergillus* species, not identified. Five of the 7 subjects with non-fumigatus *Aspergillus* infection had an

unsatisfactory response. *A. niger*, a common environmental mould, was recovered from the two subjects who had a satisfactory response to voriconazole.

In the amphotericin B arm there were 42/68 (62%) subjects who had culture proven infections due to a single *Aspergillus* species. A total of 27/42 (64%) infections were due *A. fumigatus*. A total of 5/27 (19%) subjects where *A. fumigatus* alone was recovered responded to initial amphotericin B therapy followed by EORT. In the amphotericin B arm there were 15/42 (36%) subjects with infections due to a single isolate of a non-fumigatus *Aspergillus* which included 6 *A. flavus*, 2 *A. terreus*, 3 *A. niger* and 4 *Aspergillus* species (not identified). A satisfactory response was seen in the following patients infected with a single non-fumigatus species of *Aspergillus*: 3/6 with *A. flavus*, 1/3 with *A. niger*, and 1/4 with *Aspergillus* species (not identified).

Table 22

Clinical Response in MITT Subjects and *Aspergillus* species Isolated Study 307-602

	Response to Voriconazole			Response to Amphotericin B		
Fungal Species	**Satis-factory**	**Unsatis-factory**	**Total**	**Satis-factory**	**Unsatis-factory**	**Total**
Aspergillus fumigatus	21,1↓	21	43	5	21	27
Aspergillus flavus		3,2[Y]	5	2,1[Y]↓	3,1↑	7
Aspergillus niger	1[Y],1	1	3	1	2,1[Y]	4
Aspergillus terreus		1	1		1, 1↑	2
Aspergillus nidulans	1[Y]↓		1			0
Aspergillus glacus	1[Y]		1			0
***Aspergillus* species (Not identified)**		1[Y],1	2	1	3	4

Y=patient who had the defining mould and yeast isolated from clinically relevant sites.

M=patients who had the defining mould and a non-aspergillus mould isolated from clinically relevant sites.

Z=patients who had the defining mould and a Zygomycete isolated from clinically relevant sites.

↓= MITT subjects who initially had a satisfactory response at week 12 but had an unsatisfactory response by wk 16.

↑= MITT subjects who initially had an unsatisfactory response at week 12 but had a satisfactory response by wk 16.

The most common *Aspergillus* species isolated in both treatment arms was *A. fumigatus* followed by *A. flavus, Aspergillus* species not identified, *A. niger, A. terreus, A. glacus*

and *A. nidulans*. These response rates in subjects with documented fungal infections due to a single *Aspergillus* isolate clearly demonstrate that at week 12 voriconazole is more effective in the treatment of *A. fumigatus* infections than amphotericin B.

The number of observed culture confirmed infections due to a single non-fumigatus species of *Aspergillus* were small in both treatment arms. None of the five subjects in the voriconazole arm with a single non-fumigatus *Aspergillus* isolate recovered from a clinically relevant site had a satisfactory response while 3/7 (43%) of those treated with amphotericin B had a satisfactory response.

In addition, there were 6 subjects in the voriconazole arm and 2 in the amphotericin B arm that had another fungus in addition to a non-fumigatus species of *Aspergillus* isolated from a clinically relevant site. In the voriconazole arm these included infections due to 2 *A. flavus*, 1 *A. niger*, 1 *Aspergillus* species (not identified), 1 *A. glacus* and 1 *A. nidulans*. In the amphotericin B arm there was 1 subject with *A. flavus* and 1 subject with *A. niger*.

It is well documented that infections due to non-fumigatus *Aspergillus* are increasing. As such it is important to identify appropriate therapies for the treatment of infections due to these non-fumigatus *Aspergillus* species. However, due to the low number of infections due to the non-fumigatus species of *Aspergillus* observed in studies 307/602 it is impossible to adequately determine the efficacy of voriconazole against the individual non-fumigatus species of *Aspergillus*. As a consequence, it is imperative that the sponsor continue to collect data from clinical cases to evaluate the therapeutic effect of voriconazole against these non-fumigatus species of *Aspergillus*.

The second confounding factor complicating the interpretation of the microbiologic efficacy data from studies 307/602 was the incidence of multiple fungal species recovered from clinically relevant sites. A total of 27/77 (35%) subjects in the voriconazole arm and 27/68 (40%) subjects in the amphotericin B arm had multiple fungi (yeasts and moulds) from clinically relevant sites (See table 23).

The response rates in subjects with multiple fungi from clinically relevant sites were assessed. The majority of the subjects had an *A. fumigatus* and another yeast or mould recovered from a clinically relevant site. In the amphotericin B and voriconazole arms there were 21 and 20 MITT subjects, respectively, who had more than one fungus recovered. A satisfactory response was obtained in 6/15 (40%) subjects in both treatment arms who had yeast recovered from clinically relevant sites in conjunction with *A. fumigatus*. In the voriconazole arm a satisfactory response was seen in 4/8 (50%) subjects with *C. albicans* and 0/4 subjects with *C. glabrata*. In the amphotericin B arm there were only 4 subjects with *C. albicans* and none with *C. glabrata*. One of the 4 subjects with mixed infections due to *C. albicans* responded in the amphotericin B arm. The remaining subjects had various other Candida species isolated from clinical sites, including *C. krusei*, *C. tropicalis*, *C. kyfer*, *C. inconspicum*, *Saccharomyces cerrevisia* and *Geotrichum*.

Table 23

Clinical Response in MITT Subjects with
Mixed *Aspergillus* species Isolated in Study 307-602

	Response to Voriconazole			Response to Amphotericin B		
Fungal Species	**Satis-factory**	**Unsatis-factory**	**Total**	**Satis-factory**	**Unsatis-factory**	**Total**
***A. fumigatus* and** A. flavus		1[M]	1	2		2
A. fumigatus* and *A. niger	1[Y],1		2		3	3
***A. fumigatus* and** A. terreus	1↓		1		1	1
***A. flavus* and other mould and yeast**			0	1	1	2
***A. fumigatus* and ≥1 yeast**	6	9	15	5,1↓	8,1↑	15
***A. fumigatus* and ≥1 non-fumigatus mould**		3[Z]	3	1[Z]		1
Total mixed cultures	9	13	22	10	14	24

Y=patient who had the defining mould and yeast isolated from clinically relevant sites.

M=patients who had the defining mould and a non-aspergillus mould isolated from clinically relevant sites.

Z=patients who had the defining mould and a Zygomycete isolated from clinically relevant sites.

There were 5 and 7 subjects in the voriconazole and amphotericin B arms, respectively, that had *A. fumigatus* and other moulds isolated from clinically relevant sites. Three of the 5 subjects in the voriconazole arm had a Zygomycete isolated. All 5 subjects in the voriconazole arm had an unsatisfactory response. The seven mixed mould cultures in the amphotericin B arm were due to *A. fumigatus* and 1 *A. terreus*, 2 *A. flavus* and 3 *A. niger*. One subject had *A. fumigatus* and 3 other moulds recovered from clinically relevant sites. Three of the seven amphotericin B subjects had a satisfactory response by week 12.

In both treatment arms there were a large number of subjects who had multiple fungal species recovered from clinically relevant sites. These numbers were larger than

expected. The various combinations of yeast and moulds found in the MITT patients impedes ones ability to directly compare the efficacy of voriconazole to amphotericin B in the treatment of these subjects. This is because many of the non-aspergillus fungal isolates (i.e. *C. glabrata*, Zygomycetes, *C. tropicalis)* recovered from clinically relevant sites are also know to case infections and are typically not susceptible to azole or polyene antifungal agents. However, these data do suggest that voriconazole is not effective against infections due to the Zygomycetes. It is also of interest to note that all of the *C. glabrata* co-infections did not respond to voriconazole either and that there were twice as many *C. albicans* co-infections in the voriconazole arm versus the amphotericin B arm (8 versus 4).

Susceptibility Testing:

The clinical samples obtained from subjects enrolled in studies 307 and 602 were cultured at the local laboratory. Fungi that grew were identified at the local laboratory and then shipped to the reference laboratory. For study 307, which was mostly conducted in Europe, the reference laboratory was located in the United Kingdom. For study 602, which was conducted in South, Central and North America, fungal isolates were shipped to a reference laboratory located in Richmond, Virginia in the USA.

At the reference laboratories all fungal identifications were re-confirmed and susceptibility testing was conducted. The reference laboratories conducted susceptibility testing employing the National Committee for Clinical Laboratory Standards (NCCLS) M-38P method. In these studies, amphotericin B, itraconazole and voriconazole minimum inhibitory concentrations (MIC) were determined. This portion of the review will focus on the relationship between the voriconazole and itraconazole MICs. This is because both of these antifungal agents are antifungal azoles and it is imperative to determine if cross-resistance occurs between these two agents.

Of the 145 MITT subjects with culture confirmed *Aspergillus* infections in vitro antifungal susceptibility testing was conducted on only 113 *Aspergillus* isolates. Thirty-two isolates were identified at the local laboratory and were not sent to the central laboratory for susceptibility testing. Voriconazole MICs ranged from [redacted] for all the *Aspergillus* isolates tested (See table 24). Greater than 90% of the *A. fumigatus* isolates had a voriconazole MIC of 0.125 to 0.25 µg/ml. Approximately 40% of the *A. fumigatus* isolates had identical MIC values for itraconazole and voriconazole. Itraconazole MICs were one 2-fold dilution lower than voriconazole for one half of the *A. fumigatus* isolates. Only 1 in 10 *Aspergillus fumigatus* isolates had a higher itraconazole MIC than voriconazole. These preliminary MIC results suggest that for *A. fumigatus* isolates, voriconazole MICs are equal to or one 2-fold dilution higher than for itraconazole.

For the non-fumigatus species of *Aspergillus* the voriconazole MICs ranged from [redacted] However, the vast majority of the isolates had a voriconazole MIC of 0.25-1.0 µg/ml, a 2- 4-fold increase over that found for *A. fumigatus*. When the itraconazole MICs were compared for these same isolates it was found that the voriconazole MICs were generally 2 to 4 fold higher than for itraconazole. Lastly, it is of interest to note that the 4

Zygomycetes recovered from patients enrolled in clinical trials 307 and 602 had voriconazole MIC $\geq$ 8.0 µg/ml and itraconazole MICs of $\geq$1.0 µg/ml.

Table 24

Susceptibility patterns for Aspergillus species

Recovered from MITT Subjects Enrolled in Study 307/602

Aspergillus *Species*	**Amphotericin B (MIC_{90} µg/ml)**	***Itraconazole*** **(MIC_{90} µg/ml)**	**Voriconazole (MIC_{90} µg/ml)**
A. fumigatus **n=93**	0.5-2.0	0.06-0.125	0.125-0.25
A. flavus **n=17**	1.0-4.0	0.125-0.25	0.25-0.5
A. terreus **n=4**	0.5-4.0	0.06-0.25	0.25-05
A. niger **n=12**	0.25-1.0	0.25-0.5	0.25-0.5

The ability to accurately interpret voriconazole susceptibility data obtained from clinical studies 307/602 is complicated by two issues. The first issue is that there is no approved antifungal susceptibility testing methods to evaluate antifungal agents against the filamentous fungi. The second issue is that breakpoints for the approved azoles and polyenes have not been established for the filamentous fungi. Employing the NCCLS M38-P (a proposed) method, voriconazole MICs for the various *Aspergillus* species were 2 to 4-fold higher than those found for itraconazole. Higher voriconazole and itraconazole MICs were seen for the non-fumigatus species versus *A. fumigatus* isolates. The slightly higher azole MICs seen for the non-fumigatus species versus the *A. fumigatus* isolates may explain why there were more unsatisfactory response rates seen in those subjects versus those with culture confirmed infections due to *A. fumigatus*. The 8 to 16-fold higher voriconazole and itraconazole MICs obtained with the Zygomycetes versus *A. fumigatus* also correlates with the lack of efficacy seen in the treatment of infections due to these fungi. As a phase IV commitment the sponsor should continue to collect susceptibility data from subjects with infections due to *A. fumigatus* and non-fumigatus species of *Aspergillus* to determine the overall efficacy of voriconazole against these filamentous fungi.

B. Clinical Microbiology Review of Historical Controlled Aspergillus study 150-304:

Clinical Trial Design:

Please refer to the Medical Officer's review for a detailed description of the clinical trial design.

Clinical Microbiology Results:

Study 304 was a multi-center uncontrolled study of voriconazole for the treatment of invasive aspergillosis This study was conducted in Europe where patients received voriconazole as primary or salvage therapy. One hundred thirty seven patients were enrolled into the study. Clinical samples were sent to the laboratory for histopathologic slides and/or fungal culture to confirm the presence of *Aspergillus*. Efficacy was measured as a satisfactory or unsatisfactory global response. Of the 137 subjects enrolled 75 (54%) had culture confirmed disease due to a specific *Aspergillus* species (See Table 25).

Culture confirmed cases were studied in detail to determine the efficacy of voriconazole against the various *Aspergillus* species. As seen in studies 307/602, the majority of the infections were due to *A. fumigatus*. Twenty-one of the 40 (53%) subjects with invasive disease due to *A. fumigatus* alone had a satisfactory response. A satisfactory response rate was seen in only 4/16 (25%) subjects who had *A. fumigatus* and either a yeast or mould isolated from a clinically relevant site. This reduced response rate in subjects with a mixed fungal infection was also seen in study 307/602. These data again indicate that with voriconazole it is more difficult to successfully treat subjects with multiple fungal isolates.

Table 25
Microbiology Results from Subjects Enrolled in Historical Control Study 304

Organisms Recovered from clinically Relevant Sites	Satisfactory Response	Unsatisfactory Response	Unevaluable	Total
No Microbiology Results	11	8	3	22
Yeast only	3	1	1	5
Mould not *Aspergillus*	1	0	3	4
Histopath (+) for *Aspergillus*	8	6	1	15
Non-fumigatus *Aspergillus* alone	6	13	0	19
Aspergillus Species (no ID)	9	5	2	16
A. fumigatus *alone*	21	19	0	40
A. fumigatus + mould	1	6	0	7
A. fumigatus + yeast (no ID)	3	6	0	9
Total	63	60	12	137

Clinical responses were determined for subjects with culture confirmed infections due to non-fumigatus species of *Aspergillus*. In study 304 there were 19 evaluable subjects who had infections due to non-fumigatus *Aspergillus* species. *A. flavus* was recovered from 9 subjects, *A. niger* from 4 subjects, *A. nidulans* from 4 subjects and *A. terreus* from 2 subjects (See table 26). These same Aspergillus species were also recovered from subjects enrolled in the controlled *Aspergillus* studies 307/602 with *A. flavus* being the second most common *Aspergillus* species recovered.

Table 26

Global Response Rates for Subjects with Culture Confirmed Disease due to Non-fumigatus *Aspergillus* species

Fungal Species	Satisfactory Response	Unsatisfactory Response	Total
A. terreus	0	2	2
A. flavus	2	7	9
A. niger	3	1	4
A. nidulans	1	3	4
Total	6	13	19

A satisfactory response for the non-fumigatus species of *Aspergillus* was lower than that seen for *A. fumigatus*, 6/19 (32%) versus 21/40(53%), respectively. Satisfactory responses for the individual *Aspergillus* species were observed as follows; 2/9 (22%) for *A. flavus*, 3 of 4 (75%) for *A. niger*, 1/4 (25) for *A. nidulans* and 0/2 for *A. terreus*. These responses compare to those found in the controlled clinical trial 307/602 suggesting that voriconazole is not as effective in producing a satisfactory clinical response against invasive Aspergillosis due to non-fumigatus species versus *A. fumigatus*.

The exact cause for this reduced activity against the non-fumigatus species of *Aspergillus* can not be fully explored, as susceptibility testing was not performed on the fungal isolates recovered from subjects enrolled in the study. Without susceptibility results it is impossible to determine whether drug resistance development occurred in subjects who has an unsatisfactory response or if cross-resistance was observed. However, these data support the following findings from studies 307/602: 1) voriconazole produces a satisfactory clinical outcome in 50% of the subjects who had microbiologically proven *A. fumigatus* infections, and 2) voriconazole had reduced clinical efficacy in the treatment of invasive fungal infections due to *A. flavus*, *A. nidulans* and *A. terreus*.

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Linda L. Gosey
Microbiologist (HFD 590)

Concurrences:

HFD-590/Dep Dir _____Signature_____Date

HFD-590/Micro TL_____ Signature_____Date

cc:
HFD-590/MO:Tiernan
HFD-590/CSO:Saliba
HFD-590/Chem:Seggel
HFD-590/Pharm:McMaster
HFD-590/Review Micro:Gosey

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/s/

Linda Gosey
12/12/01 03:06:01 PM
MICROBIOLOGIST

Shukal Bala
12/13/01 08:06:43 AM
MICROBIOLOGIST

Kenneth Hastings
12/14/01 07:36:40 AM
PHARMACOLOGIST

SEP 10 1999

Microbiology Review

Division of Special Pathogens and Immunologic Drug Products

(HFD-590)

IND# ———

Reviewer : Linda Gosey
Correspondence Date : 07-29-99
CDER Receipt Date : 07-30-99
Review Assigned Date: 08-06-99
Review Complete Date: 08-25-99

Sponsor: Pfizer Inc.
235 East 42nd Street
New York, New York 10017-1563

Submission Reviewed: Amendment 133

Drug Category: Antifungal

Indication: Treatment of fungal infections

Dosage Form: Oral suspension

Product Names:

a. Proprietary: Not Established

b. Nonproprietary: voriconazole; UK 109,496

Supporting Documents: None

Background and Summary:

The subject of this IND is voriconazole, an azole that is currently being assessed for its antifungal activity against numerous fungal pathogens. The information contained in this submission pertains to the sponsor's proposed changes to two clinical trials. Protocol 150-604 is an open label, non-comparative, multicenter, phase III trial that will assess the efficacy, safety and tolerability of voriconazole in the primary or secondary treatment of invasive fungal infections. Protocol 150-602 is an open label, randomized, comparative, multicenter study that will assess the efficacy, safety and

tolerability of voriconazole versus amphotericin B followed by other licensed antifungal therapy in the treatment of acute invasive Aspergillosis in immunocompromised patients.

Conclusions:

This submission contains the sponsor's proposed changes to clinical protocols 150-602 and 150-604. These changes mostly pertain to clinical parameters. However, there were two changes regarding microbiology. The first change states that fungal isolates will be sent to a reference laboratory, headed by Dr. _____ where fungal isolates will be speciated and susceptibility testing will be performed. The second microbiology protocol modification outlines how blood samples for an Aspergillus diagnostic assay will be processed. Both of these microbiology modifications to protocols 150-62 and 150-604 are acceptable.

In earlier correspondences with the sponsor additional microbiology comments and recommendations were made regarding these two protocols. Listed below are the outstanding microbiology comments that remain to be addressed by the sponsor.

Recommendations:

This submission has not addressed all the microbiology recommendations which were previously conveyed to you regarding amendments 40 and 43. Please address the following outstanding microbiology comments pertaining to clinical protocols 150-602 and 150-604:

1. Please provide the details of the laboratory protocols that will be used for microbiological measurements which include the collection and transport of clinical samples for fungal culture, the processing of specimens for the recovery of fungi and the identification of fungal isolates obtained from clinical samples taken during these clinical trials.

2. When these clinical trials are complete please provide the voriconazole MIC values for the particular fungal species isolated and a copy of the susceptibility testing method. These data may be used to help determine if voriconazole resistance develops while patients are receiving therapy.

3. Please consider conducting fluconazole and itraconazole susceptibility testing on all fungal isolates recovered from

patients enrolled in these clinical trials to determine if cross resistance occurs between voriconazole and fluconazole or itraconazole.

4. Please clarify what Aspergillus diagnostic assay will be employed in these clinical trials. Submit a copy of the Aspergillus diagnostic assay procedure/methodology.

5. Please clarify what will be done with the Aspergillus diagnostic assay results obtained from these clincal trials. Test results from non-FDA approved methods can not be used in support of the NDA.

/S/

Linda L. Gosey
Microbiologist HFD 590

Concurrences:
/S/
HFD-590/Dep Dir _____ Signature 10/20/99 Date
HFD-590/MicroTL _____ Signature 9/10/99 Date

CC:
HFD-590/ Orig.IND ——
HFD-590/ Division File
HFD-590/MO:Tierman
HFD-590/CSO:Bacho
HFD-590/Chem:Holbert
HFD-590/Pharm:McMaster
HFD-590/Review Micro:Gosey

DF

Microbiology Review

Division of Antiviral Drug Products (HFD-530)

IND# ———

Reviewer : Linda Gosey
Correspondence Date : 08-28-95
CDER Receipt Date : 08-30-95
Review Assigned Date: 09-11-95
Review Complete Date: 10-25-95

Sponsor: Pfizer Inc.
235 East 42nd Street
New York, New York 10017-1563

Submission Reviewed: Original (8-28-95)

Drug Category: Antifungal

Indication: Treatment of fungal infections

Dosage Form: Oral and IV

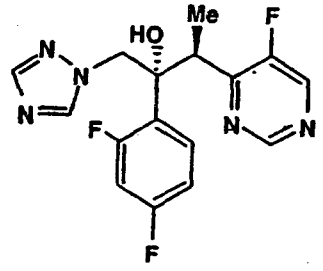
Product Names:

a. Proprietary:

b. Nonproprietary: UK 109,496

c. Chemical:

Structural Formula:



Supporting Documents:

Background:

None

Summary:

Clinical Protocol:

The sponsor has proposed a randomized, double-blinded, placebo-controlled dose escalating study to evaluate the safety, tolerance and pharmacokinetics of UK 109,496 at 200, 300 and 400 mg BID. Subjects enrolled in this study will have either acute myelogenous leukemia or be a recipient of an autologous bone marrow transplant and be at risk of developing Aspergillosis. Amphotericin B empiric therapy will be allowed in these patients for suspected, not microbiologically documented, fungal infections. The first twelve patients will receive 200 mg UK 109,496 BID for 14 days. On study days 1, 4, 7, 10, 15 and 21 blood will be collected for safety and pharmacokinetics evaluation. If the 200 mg BID dose is tolerated and there are no safety concerns then the next twelve subjects will receive the 300 mg BID dose then the 400 mg BID dose. Efficacy parameters will not be assessed in this study, therefore, with respect to microbiology this clinical trial is safe to proceed.

Preclinical Microbiology:

General Comments:

The sponsor submitted data from a number of preclinical studies that measured the antifungal activity of UK 109,496. It is well known that the in vitro MIC values for the azoles may not correlate with the in vivo activity of the agent. As a consequence, the MIC data for UK 109,496 may not be predictive of the agent's activity in humans. A number of in vivo experiments were performed to determine the antifungal activity of UK 109,496 against Aspergillus fumigatus, Cryptococcus neoformans, Candida albicans and other Candida species infections. In these studies both normal and immunosuppressed guinea pigs were infected. There are several concerns regarding the use of the guinea pig model, in particular for the Aspergillus experiments. First, mice or rabbits are the animals of choice for studying the potential antifungal activity of agents against Aspergillus infections. Second, the ability to interpret the in vivo activity of UK 109,496 using the guinea pig model is complicated by the fact that the antifungal activity of other agents, specifically the azoles, have previously been characterized in mice. The use of the guinea pig model makes it impossible to compare the antifungal activity of UK 109,496 to other agents where a different animal

species was employed. Third, the sponsor states that UK 109,496 is poorly absorbed by rats and mice, as a consequence, they chose to use guinea pigs to assess antifungal activity. This statement is in direct conflict with the pharmacology/toxicology data where rodent models were chosen to assess the toxicity profile of UK 109,496. In the safety meeting Dr. Farrely, the pharmacology supervisor, stated that UK 109,496 was extensively metabolized in the rodent model and was toxic and teratogenetic. If in reality the compound could not be absorbed by rodents then the pharmacology/toxicology studies should have been performed in a different animal model and toxicity would not have been observed. This information leads the reviewing microbiologist to suspect that the sponsor did not want to use a murine model to assess the antifungal activity of UK 109,496 due to the toxicity profile and the possibility that activity might not have been demonstrated in this model. Lastly, in most of the in vivo studies the sponsor used amphotericin B as an active control agent. Currently, amphotericin B is the most effective agent against most fungal infections due to its cidal activity. However, in virtually all of the experiments conducted by the investigator, amphotericin B was inactive against Aspergillus, Candida and Cryptococcal guinea pig infections. These data are inconsistent with other preclinical studies. These inconsistencies make the reviewing microbiologist question the validity of all of the preclinical data.

With respect to the guinea pig experiments there are additional concerns regarding the design of the treatment studies and the interpretation of the data. 1). In all of the Aspergillus in vivo studies, guinea pigs were treated 1-4 hours post infection. This infection model more closely resembles a prophylactic study as opposed to an established infection model. Studying the activity of UK 109,496 against established diseases would have more closely resembled the actual infection state that will be treated in humans. 2). In all of the in vivo experiment conducted by the investigator guinea pigs were only treated for 5 days. This is an unusually short period of time to assess activity. In addition, it does not address the issue of long term therapeutic response, post antibiotic effect, rate of relapse and drug resistance development. 3). In the guinea pig infection model the investigator chose to necropsy animals 16 hours after the last therapeutic dose. Again, by necropsying animals soon after the last dose one can not determine the post antibiotic effect of the agent or the rate of relapse. As a consequence, the ability to fully characterize the antifungal activity of UK 109,496 is limited. In an effort to supply the medical officer with as much preclinical activity information as possible, the reviewing microbiologist will summarize the data from the in vitro and in vivo studies. However, care should be taken when interpreting the data due to the concerns mentioned above.

In vitro Studies:

The in vitro activity of UK 109,496 was measured by the agar

Isolates of different fungal species were inoculated onto agar plates containing various concentrations of drug using a ——— inoculator. Plates were incubated at —— for —— hours. For most fungal species tested, specifically Aspergillus species, the MIC values for UK 109,496 were comparable to itraconazole and lower than fluconazole MIC values (See table 1). UK 109,496 had lower MIC values than itraconazole for Scedosporium inflatum, Fusarium species, and Pseudallescheria boydii. However, for Mucor species and Candida krusei itraconazole MIC values were lower than UK 109,496.

Comments: There are several reasons why caution should be used when interpreting the in vitro data. First, in this study the investigator did not use the recommended NCCLS fungal susceptibility testing method. Second, MIC values were determined at —— hours which is too early for the molds, specifically Aspergillus species, the dermatophytes and the dematiaceous molds, which take approximately 5 days for initial growth to appear. Third, an incubation temperature of ——— is not the optimal temperature for most of the fungi tested. Generally, molds grow better at 27° C. Therefore, the in vitro data may not accurately reflect the true antifungal activity of UK 109,496.

Table 1

Table 1 *In vitro* activity of UK-109,496, itraconazole, fluconazole and amphotericin B against various fungal pathogens

Organism	MIC (µg/ml)			
	UK-109,496	Itraconazole	Fluconazole	Amphotericin B
Aspsergillus fumigatus	0.35 ± 0.08 (39)	0.39 ± 0.4 (38)	>100 (39)	0.05 ± 0.54 (36)
Aspergillus flavus	0.58 ± 0.29 (21)	0.15 ± 0.18 (21)	>100 (21)	1.75 ± 2.5 (21)
Aspergillus niger	0.39 ± 0.38 (14)	0.23 ± 10.3 (14)	>100 (14)	0.7 ± 0.56 (14)
Aspergillus terreus	0.50 ± 0.22 (11)	0.11 ± 0.1 (11)	>100 (11)	1.56 ± 0.7 (11)
Aspergillus nidulans	0.19 (3)	0.21 ± 0.06 (3)	>100 (3)	1.8 ± 1.0 (3)
Fusarium solani	4.0 ± 1.8 (3)	>100 (3)	>100 (3)	1.2 ± 6.9 (3)
Fusarium oxysporum	1.1 ± 0.55	>100 (2)	>100 (2)	1.1 ± 0.55 (2)
Acremonium kilensii	0.39 (2)	>100 (2)	>100 (2)	3.1 ± 3.3 (2)
Pseudallescheria boydii	0.39 (2)	4.5 ± 2.26 (2)	25 (2)	>100 (2)
Exophiala dermatidis	0.02 (1)	0.02 (1)	6.3 (1)	0.05 (1)
Hendersonula toruloidea	0.09 ± 0.04 (6)	50 ± 40.6 (6)	25 ± 24.6 (6)	0.03 ± 0.01 (6)
Mucor javanicus	50 (1)	12.5 (1)	>100 (1)	0.02 (1)
Mucor genevensis	100 (1)	50 (1)	>100 (1)	0.02 (1)
Mucor rouxii	6.3 (1)	0.78 (1)	>100 (1)	0.02 (1)

Continued.....

Organism	MIC (µg/ml) UK-109,496	Itraconazole	Fluconazole	Amphotericin B
Candida albicans	0.04 ± 8.18 (263)	0.03 ± 3.7 (219)	3.5 ± 38.9 (263)	0.08 ± 0.06 (262)
Candida glabrata	0.42 ± 1.4 (94)	1.3 ± 26.9 (80)	18 ± 63.2 (94)	0.11 ± 0.05 (94)
Candida krusei	0.32 ± 0.15 (31)	0.04 ± 0.07 (27)	46 ± 22.2 (31)	0.11 ± 0.09 (31)
Candida parapsilosis	0.01 ± 0.02 (23)	0.02 ± 0.01 (23)	1 ± 1.1 (23)	0.03 ± 0.04 (23)
Candida tamata	0.05 ± 0.03 (3)	0.02 ± 0.1 (3)	11.5 ± 6.3 (3)	0.06 ± 0.02 (3)
Candida kefyr	0.001 ± 0.03 (8)	0.01 ± 0.03(7)	0.22 ± 2.1 (8)	0.05 ± 0.12 (8)
Candida tropicalis	0.03 ± 0.08 (34)	0.01 (29)	1.4 ± 2.2 (34)	0.03 ± 0.04 (34)
Candida guilliermondii	0.05 ± 0.14 (6)	0.01 ± 0.01 (6)	3.8 ±1.6 (6)	0.03 ± 0.01 (6)
Cryptococcus neoformans	0.23 ± 0.63 (179)	0.11 ± 6.2 (155)	17.5 ± 22.8 (179)	0.07 ± 0.08 (179)
Cryptococcus albidus	0.39 (3)	0.40 (3)	46 ± 7.2 (3)	0.12 (3)
Trichosporon beigelii	0.09 (2)	0.02 (2)	2.2 (2)	0.09 (2)
Trichosporon cutanaeum	0.19 (2)	0.07 (2)	3.1 (2)	0.04 (2)
Trichosporon capitatum	0.19 (8)	0.03 (8)	10 (8)	0.14 (8)
Trichosporon penicillatum	0.60 (6)	0.50 (6)	50 (4)	0.19 (6)
Rhodotorula glutinis	12.5 (3)	Not tested	>100 (3)	0.11 ± 0.06 (3)
Geotrichum candidum	0.39 (4)	0.39 (4)	50 ± 12.5 (4)	0.19 (4)
Hansenula wingei	0.01 (1)	0.01 (1)	0.39 (1)	0.05 (1)
Saccharomyces cerevisiae	0.10 (1)	0.39 (1)	6.30 (1)	0.10 (1)
Trichophyton mentagrophytes	0.32 (13)	0.13 (13)	200 (13)	0.32 (13)
Trichophyton quinckeanum	0.05 (3)	0.02 (3)	25 (3)	0.64 (3)
Trichophyton rubrum	0.09 (28)	0.05 (28)	31 (28)	0.32 (28)
Microsporum gypseum	0.39 (2)	0.55 (2)	100 (2)	0.28 (2)
Paecilomyces lilacinus	0.78 ± 0.8 (2)	not tested	>100 (2)	>100 (2)
Monascus ruber	100 (8)	not tested	>100 (3)	0.13 ± 0.1 (3)
Scedosporium inflatum	3.1 (1)	>100 (1)	> 100 (1)	>100 (1)

MICs were determined by agar dilation in _____ medium, see Methods Appendix, Section 4.1.

Values are means, ± S.D., of results obtained from the number of isolates shown in parentheses
( ) = Number of isolates

In vivo Studies:

Aspergillus fumigatus infections:

Normal and immunosuppressed guinea pigs were IV infected with 10^7 and 10^6 Asp. fumigatus spores, respectively. Untreated control animals were dead by day 5. Treatment started 1 hour post and 4 hours post infection in the normal and immunosuppressed animals, respectively. UK 109,496 at 1, 5 or 10 mg/kg was administered BID via gavage for 5 days. Sixteen hours after the last dose animals were sacrificed. Fungal burden was assessed in kidney or liver tissue in normal and immunosuppressed guinea pigs, respectively. UK 109,496 at 5 and 10 mg/kg protected 100% of the normal and immunosuppressed guinea pigs. Kidney and liver tissue was sterilized in all normal and immunosuppressed animals receiving 10 mg/kg UK 109,496. The 5 mg/kg dose decreased fungal burden by three logs in the kidney and liver of normal and immunosuppressed guinea pigs, respectively (See tables 2 and 3).

Table 2

Table 2 Activity of UK-109,496, itraconazole, fluconazole and amphotericin B against systemic aspergillosis in immune normal guinea pigs

Compound	Dose (mg/kg)	Survivors	Cures	*Aspergillus* content of kidney (CFU/g homogenate)
UK-109,496	10	15/15	15/15	0.00***
	5	15/15	12/15	0.10***
	1	8/15	4/15	1.90 ± 1.47
Itraconazole	10	15/15	9/15	0.31***
	5	13/15	7/15	0.39***
	1	6/15	2/15	1.39 ± 1.29
Fluconazole	50	12/15	1/15	2.60 ± 1.05
Amphotericin B	4	3/5	0/5	2.95 ± 0.75
Controls	0	0/13	0/13	3.21 ± 0.31

Animals were dosed orally b.i.d. for 5 days except for amphotericin B which was administered i.p. o.d. for 5 days, commencing 1h post-infection. The vehicle was 0.5ml polyethylene glycol 200
Kidneys were harvested 16h after the last dose

Values are means, ± S.D., of the results obtained from 3 separate experiments with groups of 5 animals, except where cures were observed, when an average value only is given

CFU/g = $\log_{10}$ colony-forming units per g of kidney homogenate

Significance of difference from vehicle group (Student's independent t-test) ***P <0.001

Table 3

Table 3 Activity of UK-109,496, itraconazole, fluconazole and amphotericin B against systemic aspergillosis in cyclophosphamide- and dexamethasone-immunocompromised guinea pigs

Compound	Dose (mg/kg)	Survivors	Cures	*Aspergillus* content of liver (CFU/g tissue)
UK-109,496	10	27/28	28/28	0.00***
	5	28/28	14/28	0.32***
	2.5	8/10	0/10	1.70 ± 0.10**
	1	4/28	0/28	3.26 ± 0.44
Itraconazole	10	27/27	4/27	0.66 ± 0.58***
	5	21/28	2/28	2.39 ± 0.65
	1	3/25	0/25	3.30 ± 0.39
Fluconazole	20	3/17	0/17	3.21 ± 0.32
Amphotericin B	4	3/9	0/9	3.26 ± 0.64
Vehicle	0	1/25	0/25	3.19 ± 0.40

Animals were immunocompromised with 2mg/kg p.o. dexamethasone daily, starting 4 days prior to infection plus two 100mg/kg i.p. doses of cyclophosphamide 4 days and 1 day prior to infection

Animals were dosed orally b.i.d. for 4 days except for amphotericin B which was administered i.p. o.d. for 4 days, commencing 4h post-infection. The vehicle was 0.5ml polyethylene glycol 200

Livers were harvested 16h after last dose

Values are means, ± S.D., of the results obtained from 3 separate experiments with groups of 6 to 8 animals, except when cures were observed, when an average value only is given

CFU/g = $\log_{10}$ colony-forming units per g of liver

Significance of difference from vehicle group (Student's independent t-test) ** P <0.01, *** P <0.001

Immunosuppressed guinea pigs were intratracheally infected with 5 x 10^7 Asp. fumigatus spores. UK 109,496 at doses of 2, 4 and 8 mg/kg were administered BID starting 24 hours post infection for a total of 7 days. Lung tissue was harvested 16 hours after the last dose of drug and cultured for fungi. UK 109,496 at 4 and 8 mg/kg reduced fungal burden in lung by 1.8 and 1.5 logs, respectively (See table 4).

Table 4

Table 4 Activity of UK-109,496, itraconazole and fluconazole against pulmonary aspergillosis in cortisone acetate-immunocompromised guinea pigs

Compound	Dose (mg/kg)	Survivors	Cures	*Aspergillus* content of lung (CFU/g tissue)
UK-109,496	8	22/22	11/22	0.21*** ###
	4	18/20	6/20	0.46*** ###
	2	14/14	2/14	0.89 ± 0.91
Itraconazole	8	18/18	2/18	1.24 ± 1.05
	4	17/18	2/18	1.15 ± 0.95
Fluconazole	50	17/17	3/17	1.02 ± 0.98
Vehicle	0	16/19	0/19	2.01 ± 1.21

Animals were immunocompromised with six 100mg/kg s.c. doses of cortisone acetate 7, 4 and 2 days prior to infection, on the day of infection, and 3 and 5 days post-infection

Animals were dosed orally b.i.d. for 7 days, commencing 24h post-infection. The vehicle was 0.5ml polyethylene glycol 200

Lungs were harvested 16h after last dose

Values are means, ± S.D., of results from 3 separate experiments with groups of 6 to 8 animals, except where cures were observed, when an average value only is given

CFU/g = $\log_{10}$ colony-forming units per g of lung.

Significance of difference from vehicle group (Student's independent t-test) *** P <0.001

Significance of difference from itraconazole group (Student's independent t-test) ### P <0.001

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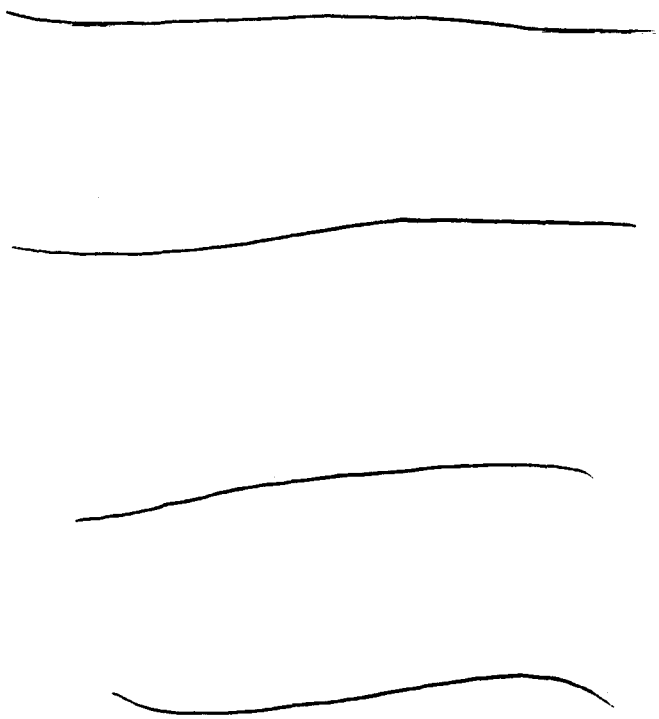
Table 9



Cryptococcus neoformans infections:

Normal guinea pigs were intratracheally infected with 10^5 Cr. neoformans yeast cells which produced localized pulmonary infection. Twenty four hours post infection 21 days of antifungal therapy commenced. At the end of treatment surviving mice were necropsied and lung tissue removed. All animals survived in all treatment groups at all doses. However, when UK 109,496 was administered at 10 and 20 mg/kg BID the fungal burden in lung was reduced by 2 log over untreated control animals (See table 10).

Table 10

Table 10 Activity of UK-109,496, itraconazole, fluconazole and amphotericin B against pulmonary cryptococcosis in immune normal guinea pigs

Compound	Dose (mg/kg)	Survivors	*Cryptococcus* content of lung (CFU/g tissue)
UK-109,496	20	14/14	2.49 ± 0.65***
	10	15/15	2.72 ± 0.75***
	2.5	16/16	4.37 ± 0.74
Itraconazole	20	16/16	2.64 ± 0.71***
	10	16/16	2.21 ± 0.65***
	2.5	13/13	3.18 ± 0.63*
Fluconazole	20	16/16	2.19 ± 0.56***
	10	15/15	2.24 ± 0.69***
	2.5	15/15	3.14 ± 1.01*
Amphotericin B	5	16/16	3.09 ± 1.23
Controls	0	16/16	4.82 ± 0.50

Animals were dosed orally b.i.d. for 21 days except for amphotericin B which was administered i.p. o.d. on alternate days, commencing 24h post-infection. The vehicle was 0.5ml polyethylene glycol 200

Lungs were harvested 16h after last dose

Values are means, ± S.D., of the results obtained from 3 separate experiments with groups of 5 or 6 animals

CFU/g = $\log_{10}$ colony-forming units per g of lung.

Significance of difference from vehicle group (Student's independent t-test) * P <0.05 *** P <0.001

4.2.8 Intracranial cryptococcosis in immune normal guinea pigs

After intracranial infection with 5 x 10^2 *C. neoformans* yeasts, animals received oral therapy for 9 or 10 days. Compound efficacy was measured as a reduction of the brain fungal load. UK-109,496 (1 to 10mg/kg p.o. b.i.d. for 10 days) reduced the mean, ± S.D., brain fungal load in a dose-dependent way, and at 10mg/kg p.o. b.i.d. for 10 days it was reduced from 6.26 ± 0.24 to 2.01 ± 0.66CFU/g (Table 11). This reduction was comparable with the inoculum level and in keeping with the fungistatic mode of action of UK-109,496 against *Cryptococcus*. Itraconazole and fluconazole at 10mg/kg p.o. b.i.d. for 10 days, gave efficacies similar to that of UK-109,496 at the same dose level, reducing the brain fungal load to 1.52 ± 0.61 and 1.11 ± 0.28CFU/g, respectively. In contrast, UK-109,496 was less active than itraconazole or fluconazole at dose levels below 10mg/kg. Thus, at the lowest dose of 1mg/kg p.o. b.i.d. for 10 days, the brain fungal loads for UK-109,496, itraconazole and fluconazole were 5.85 ± 0.55, 4.68 ± 0.93 and 3.90 ± 0.43CFU/g, respectively. However, when administered in a t.i.d. regimen (2 to 3mg/kg p.o. t.i.d.

Intracranial infections were also established in guinea pigs with a 5 x 10^2 inoculation of Cr. neoformans spores. Therapy was administered BID or TID for 10 days starting 1 hour post infection. When UK 109,496 was administered at 5 and 10 mg/kg BID the fungal burden in brain tissue was reduced by 2 1/2 and 4 logs, respectively (See table 11). UK 109,496 at 8 mg/kg TID decreased viable yeast in brain tissue by 3 1/2 log (See table 12).

Table 11

Table 11 Activity of UK-109,496, itraconazole and fluconazole against intracranial cryptococcosis in immune normal guinea pigs

Compound	Dose (mg/kg)	*Cryptococcus* content of brain (CFU/g tissue)
UK-109,496	10	2.01 ± 0.66***
	5	3.51 ± 0.59***
	1	5.85 ± 0.55
Itraconazole	10	1.52 ± 0.61***
	5	1.73 ± 0.57***
	1	4.66 ± 0.93*
Fluconazole	10	1.11 ± 0.28***
	1	3.90 ± 0.43***
Amphotericin B	2	6.01 ± 0.21
Vehicle	0	6.26 ± 0.24

After intracranial infection, animals received oral therapy 24h post infection and then b.i.d. for 9 days, except for amphotericin B which was administered i.p. on alternate days, commencing 1h post-infection. The vehicle was 0.5ml polyethylene glycol 200

Brains were harvested 16h after last dose

Values are means, ± S.D., of the results obtained from 2 separate experiments with groups of 6 animals

CFU/g = $\log_{10}$ colony-forming units per g of brain

Significance of difference from vehicle group (Student's independent t-test) * P <0.05 *** P <0.001

Table 12

Table 12 Activity of UK-109,496 and fluconazole against intracranial cryptococcosis in immune normal guinea pigs

Compound	Dose (mg/kg)	*Cryptococcus* content of brain (CFU/g tissue)
UK-109,496	8 t.i.d.	1.43 ± 0.34***
	6 t.i.d.	2.18 ± 0.36***
	4 t.i.d.	1.92 ± 0.33***
	2 t.i.d.	3.56 ± 0.17***
Fluconazole	5 b.i.d.	1.30 ± 0.18***
	1 b.i.d.	3.95 ± 0.62*
Vehicle	0	5.96 ± 0.49

Animals received oral therapy starting 3h after infection and then b.i.d. or t.i.d. for 9 days. The vehicle was 0.5ml polyethylene glycol 200

Brains were harvested 8 to16h after last dose

Values are means, ± S.D., of the results obtained from 2 separate experiments with groups of 6 animals

CFU/g = $\log_{10}$ colony-forming units per g of brain.

Significance of difference from vehicle group (Student's independent t-test) * P <0.05 *** P <0.001

Conclusions:

UK 109,496 is a new antifungal azole that is currently being evaluated for its activity against several fungal pathogens. In this submission the sponsor has proposed a randomized, double-blinded, placebo-controlled dose escalating study to evaluate the safety, tolerance and pharmacokinetics of UK 109,496 at 200, 300 and 400 mg BID. Subjects enrolled in this study will have either acute myelogenous leukemia or be a recipient of an autologous bone marrow transplant as well as be at risk of developing Aspergillosis. Because this is a phase I study, efficacy parameters will not be assessed. Therefore, with respect to microbiology there are no safety concerns regarding the proposed clinical trial.

A number of preclinical studies were conducted measuring the antifungal activity of UK 109,496. However, the interpretation of the data are difficult and complicated by a number of issues. With resect to the in vitro data, it is well known that MIC values for antifungal azoles do not necessarily correlate with the in vivo activity of the agent. In addition, the investigator chose not to use the proposed NCCLS methodology for determining UK 109,496 MIC values. Instead they used an unfamiliar medium, and determined all MIC values at 48 hours. This incubation time is extremely short particularly for the molds. As consequence, the MIC data for UK 109,496 may not be predictive of the agent's actual activity in humans.

A number of in vivo experiments were also performed to determine the antifungal activity of UK 109,496 against Aspergillus fumigatus, Cryptococcus neoformans, Candida albicans and other Candida species infections using the guinea pig model. The sponsor states that the guinea pig model was chosen for the activity studies because UK 109,496 is poorly absorbed by rats or mice. However, the pharmacology/toxicology studies were conducted in rodents. In the safety meeting Dr. Farrely, the pharmacology supervisor, stated that UK 109,496 was extensively metabolized in the rodent model and was toxic and teratogenetic. In describing the toxicity data there was no indication that the drug was not absorbed by rodents. This information leads the reviewing microbiologist to suspect that the sponsor did not want to use a murine model to assess the antifungal activity of UK 109,496 because of the toxicity profile or possibly because activity may not have been demonstrated in a rodent model.

With respect to the individual guinea pig experiments there are additional concerns regarding the design of the treatment studies and the interpretation of the data. 1). In all of the Aspergillus in vivo studies, guinea pigs were treated 1-4 hours post infection. This type of infection model would more closely mimic a prophylactic treatment study. The activity of UK

109,496 was not studied against established disease, which is one of the proposed indications for human use. 2). The treatment studies were conducted for an unusually short period of time of only 5 days. Long term therapeutic response, post antibiotic effect, rate of relapse and drug resistance development were not assessed. 3). Necropsies were only conducted at one time point, 16 hours after the last therapeutic dose. By only performing necropsying on animals soon after the last dose one can not determine the rate of response, the post antibiotic effect of the agent or the rate of relapse. 4). In most of the in vivo studies the sponsor used amphotericin B as an active control agent. However, in virtually all of the experiments conducted by the investigator amphotericin B appeared to be inactive against Aspergillus, Candida and Cryptococcal infections in the guinea pig model. This raises great concern, because these data are inconsistent with other preclinical studies where amphotericin B has been shown numerous times to be the most active antifungal agent. As a consequence, the ability to fully characterize the antifungal activity of UK 109,496 is limited. While the data from the in vitro and in vivo studies suggest that UK 109,496 is active against Aspergillus fumigatus, Candida albicans, fluconazole resistant Candida albicans, Candida krusei, Candida glabrata and Cryptococcus neoformans organisms caution should be used when interpreting the data due to the concerns mentioned above.

Recommendations:

At this time there ar no microbiology comments to be conveyed to the sponsor.

/S/

Linda L. Gosey
Microbiologist

Concurrences:

HFD-530/Dep Dir _ /S/ ___Signature 11-11-85 Date

HFD-530/Smicro___ __ Signature 11/14/85 Date

CC:

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